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(54) Title: MONOCLONAL ANTIBODIES TO LEUKOCYTE ADHESION MOLECULE-1

(57) Abstract

A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-1, -2, -4, -5, -6, -7, -8, -9, -10, -11, -14 or -15; the hybridoma cell which produces such monoclonal antibody; and methods of using such monoclonal antibody.

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MONOCLONAL ANTIBODIES TO LEUKOCYTE
ADHESION MOLECULE-1

5   This application is a continuation-in-part of Tedder
et al., U.S.S.N. 07/720,602, filed June 25, 1991, and
Tedder, U.S.S.N. 07/700,773, filed May 15, 1991, both of
which are continuations-in-part of Tedder, U.S.S.N.
07/313,109, filed February 21, 1989, each of which is herein
10   incorporated by reference.

15   This invention relates to human leukocyte-associated
cell surface proteins, and monoclonal antibodies specific
for such proteins.

Background of the Invention

Genes exclusively expressed by one cell lineage, but
20   not by others, often define the function of that cell
population. The generation of genes by the assembly of
functionally independent domains has occurred frequently as
new genes have evolved to encode proteins with new
functions. An inducible endothelial-leukocyte adhesion
25   molecule (ELAM-1), having several functionally independent
domains, is expressed on the surface of cytokine-treated
endothelial cells. This molecule is thought to be
responsible for the accumulation of blood leukocytes at
sites of inflammation by mediating the adhesion of cells to
30   the vascular lining (Bevilacqua et al., Proc. Natl. Acad.
Sci. USA 84:9238 (1987)). A granule membrane protein found

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done by any method or combination of methods for such
determinations generally known to immunologists, including
the following:

1.   A recombinant gene encoding a chimeric protein composed

5   of one of the LAM-1 functional domains (e.g., the lectin
domain) fused to the other domains (e.g., the EGF-like and
SCR domains) from another selectin could be used to assay
for binding by the stated anti-LAM1 mAb and by the antibody
to be tested. If one binds to the chimeric protein while

10   the other does not, the two mAbs have different epitopes.

2.   An antibody binding to its epitope can block the
subsequent binding of a different antibody recognizing the
same or a physically proximal epitope. Thus, if a first
antibody bound to its epitope blocks the binding of a second

15   antibody, the two are likely to recognize the same epitope.

3.   LAM-1 functions are carried out by specific regions of
the molecule, which are composed of individual epitopes.
Therefore, an epitope involved in ligand binding, for
example, can be identified by the ability of an antibody

20   bound to that epitope to inhibit a given LAM-1 function,
such as the binding of LAM-1 to PPME, fucoidin, HEV, or
activated endothelial cells. Also, the binding of an
antibody to its epitope may completely or only partially
inhibit the given LAM-1 function, depending on the proximity

25   of the epitope to the ligand binding site of the protein, so
that the epitopes of two different antibodies may be
distinguished on the basis of the difference in degree of
inhibition by the two antibodies.

4.   Some epitopes of LAM-1 may also be involved in the

30   regulation of function of the molecule, such that antibody
binding to those epitopes may augment a given LAM-1
function.   For example, antibody binding to the LAM1-1 or

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expressing LAM-1; contacting the cells with a reagent which
includes the mAb; and determining which cells form an immune
complex with the reagent. This reagent may be the mAb alone
(e.g., in the form of a purified preparation or

5   unfractionated ascites fluid), or may be the mAb conjugated
with a detectable label (such as a detectable enzyme,
fluorophore, or radioactive moiety). Determination of which
cells form an immune complex with the reagent may be
accomplished by any of a variety of standard immunological

10 techniques, including immunoprecipitation, indirect or
direct immunofluorescence with flow cytometry analysis, or
immunosorbent assays.

       In addition, the mAb of the invention may be used in
a method of isolating cells expressing LAM-1, which method

15 includes the steps of providing a sample of cells, at least
some of which are suspected of expressing LAM-1; contacting
the cells with the mAb or a reagent including the mAb; and
separating those cells which have formed an immune complex
with the mAb, from those cells which have not. This

20 separation may be performed by any of a variety of standard
immunological techniques, including fluorescence-based cell
sorting, magnetic bead-based separation protocols, and the
use of solid phase bound antibody.

       The mAb of the invention is also useful in a

25 diagnostic assay for detecting leukocyte activation in an
animal, which activation may be attributable, for example,
to inflammation, an autoimmune response, or rejection of an
organ or tissue transplant. This method is accomplished by
obtaining a fluid sample from an animal (preferably a

30 human), which sample may be, for example, blood, serum,
plasma, saliva, tears, cerebral spinal fluid, or urine;
contacting the sample with the mAb of the invention; and
detecting (by standard immunological techniques) formation

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recipient, which method includes administering to the
patient a therapeutic agent including a therapeutic amount
of the LAM-1 protein or a domain thereof, or of an
antagonist to the LAM-1 protein or domain thereof, or of a
5   fusion protein including the LAM-1 protein or a domain
thereof covalently bonded to an immunoglobulin heavy chain
constant region. The therapeutic agent is administered in a
pharmaceutically acceptable carrier substance.

In another aspect, the invention features using the
10   LAM-1 protein or domain thereof to identify a ligand that
binds to the protein or to a molecule that is specifically
associated with the protein, or fragment thereof, to
generate a functional molecule. Ligands so identified can
also be used in the methods of the invention described
15   above.

As used herein the term "antagonist to LAM-1"
includes any agent which interacts with LAM-1 and interferes
with its function, e.g., antibody reactive with LAM-1 or any
ligand which binds to LAM-1. The term "identify" is
20   intended to include other activities that require
identification of an entity, such as isolation or
purification. The term "essentially purified" refers to a
protein or nucleic acid sequence that has been separated or
isolated from the environment in which it naturally occurs.
25   Leukocyte-associated cell surface protein LAM-1
plays an important role in leukocyte-endothelial cell
interactions, especially selective cell trafficking to sites
of inflammation. The LAM-1 protein or domains thereof, or
other molecules that interfere with leukocyte adhesion and
30   function, can be used therapeutically to inhibit the
inflammatory response and to treat such conditions as tissue
damage and metastasis of cancer cells.

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intensity of staining with each mAb was rated on a (+) to
(++++) scale as shown on a three decade log scale. The
background staining obtained using an unreactive isotype-
matched mAb was less than 5% as shown by the shaded
5   histograms in the first panels.

        Fig. 10 depicts modulation of PPME binding by anti-
LAM-1 mAb. Flow cytometry analysis of PPME-FITC binding to
human blood mononuclear cells after treatment of the cells
with medium or anti-LAM-1 mAb 20 min prior to PPME-FITC
10   staining. PPME staining is completely inhibited by 5 mM
EDTA (shaded panel), indicating that all of the PPME binding
by PBMC is due to LAM-1. This is confirmed by the
observation that inhibition by LAM1-3 also reduces the level
of PPME staining to background.

15   Fig. 11 depicts evolutionary conservation of the
LAM1-3 epitope. Blood mononuclear cells from a human, cow,
rhesus monkey, dog, cat and rabbit were examined in indirect
immunofluorescence assays for expression of the LAM-1
epitope identified by the anti-LAM1-3 mAb (dark line). The
20   fluorescence histogram of cells treated with an unreactive
murine IgG$_1$ mAb are also shown (thin line). Cells were
examined by flow cytometry analysis and relative
fluorescence intensity of staining is shown on four or three
decade scales as indicated. Cell samples were examined at
25   different times with different flow cytometer settings so
that individual histograms are not directly comparable.

        Fig. 12 depicts conservation of the PPME binding
receptor by human, tamarin, dog, and rabbit blood
mononuclear cells. Cells were incubated with PPME-FITC
30   (3 μg/ml) in medium, medium containing saturating
concentrations of the anti-LAM1-3 or anti-LAM1-4 mAb or
medium containing 5 mM EDTA. Relative fluorescence

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al., Eur. J. Immunol. 20:1351, 1990) and that of neutrophil
LAM-1 is 90-100,000 (Kansas et al., J. Immunol. 142:3058,
1989). This presumably results from different patterns of
post-translational processing as occurs with many molecules
5   expressed by both cell lineages. LAM-1 affinity for ligand
is transiently increased following leukocyte activation with
lineage-specific agents, which may partially explain the
differences in migration between neutrophils and lymphocytes
(Spertini et al., Nature 349:691, 1991). Among lymphocytes,
10 LAM-1 is expressed by the majority of circulating T and B
cells, is lost following mitogen stimulation, but is found
on some antigen-specific memory T cells (Tedder et al., Eur.
J. Immunol. 20:1351, 1990; Kanof et al., J. Immunol.
140:3701, 1988). LAM-1 is a member of the selectin family
15 of cellular adhesion/homing receptors, which play important
roles in leukocyte-endothelial cell interactions, especially
selective cell trafficking to sites of inflammation. Like
other members of this family, LAM-1 contains an amino-
terminal lectin-like domain, followed by an epidermal growth
20 factor (EGF)-like domain and short consensus repeat units
(SCR) similar to those found in C3/C4 binding proteins. The
lectin domain appears to interact with specific
glycoconjugates expressed on high endothelial venules (HEV)
of peripheral lymph nodes. This interaction is calcium
25 dependent and can be inhibited by mannose 6-phosphate or
mannose 6-phosphate rich polysaccharides, such as the
phosphomannan mcnoester fragment, PPME. PPME is
particularly useful as a soluble ligand since it can be
labelled and used to assess LAM-1 binding activity without
30 the influence of other adhesion molecules involved in
leukocyte binding (Spertini et al., Nature 349691, 1991;
Spertini et al., Leukemia (in press) 1991).

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occurred. One of the 261 RAJI+ HSB2- cDNA clones isolated,
B125, contained a 1.90 kb cDNA insert that hybridized with a
2.4 kb RNA species found in several B cell lines (Tedder et
al., supra). However, B125 did not hybridize with any of
the other RAJI+ RSB2- clones or with mRNA from several T
cell lines. The B125 cDNA clone was characterized by
restriction mapping and nucleotide sequence determination.
A near-full-length 2.3 kb cDNA that hybridized with B125 was
isolated, sequenced, and termed pLAM-1.

As shown in Fig. 1A, a restriction map was
constructed by the standard single, double or triple
digestions of pLAM-1. The coding region is shown in black.
Arrows indicate the direction and extent of nucleotide
sequence determination and the open circles indicate 5'-end
labeling. In Fig. 1B, a schematic model of the structure of
the LAM-1 mRNA is shown. Thin lines indicate 5' and 3'
untranslated sequences (UT), while the thick bar indicates
the translated region. The boxes represent the lectin-like
and epidermal growth factor (EGF)-like domains and the two
short consensus repeat (SCR) units. The open box indicates
the transmembrane (TM) region.

pLAM-1 contains a 1,181 bp open reading frame that
could encode a protein of 372 amino acids, as shown in
Fig. 2. The numbers shown above the amino acid sequence
designate amino acid residue positions. The numbers to the
right indicate nucleotide residue positions. Amino acids
are designated by the single-letter code, and * indicates
the termination codon. The boxed sequences identify
possible N-linked glycosylation sites. Hydrophobic regions
that may identify signal and transmembrane peptides are
underlined. The amino acid sequence of LAM-1 indicates a
structure typical of a membrane glycoprotein. The mature
LAM-1 protein has an extracellular region of about 294 amino

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263:9557 (1988)). The lectin domain includes amino acid
residues 42-170 given in Fig. 2.

The next domain of 36 amino acids, at residues 171-
206 shown in Fig. 2, is homologous (36-39%) with epidermal
5   growth factor (EGF) (Gregory, Nature 257:325 (1975)) and the
EGF-like repeat units found in Factor IX (Yoshitake et al.,
Biochem. 25:3736 (1985)) and fibroblast proteoglycan core
protein (Krusius et al., supra) (Fig. 3B).

Immediately following these domains are two tandem
10   domains of 62 amino acids each, shown as residues 207-269
and 270-331 of Fig. 2, that are homologous with the short
consensus repeat unit (SCR) that comprises the IL-2 receptor
(Leonard et al., Nature 311:626 (1984)), Factor XIII
(Ichinose et al., Biochem. 25:4633 (1986)) and many C3/C4
15   binding proteins (Klickstein et al., J. Exp. Med. 165:1095
(1987); and Morley et al., EMBO J. 3:153 (1984)). In
contrast with all of the previously described SCR that
contain four conserved Cys residues, each of these two SCR
possesses six Cys residues. The four conserved Cys residues
20   found in all SCR are indicated in Fig. 3C by (*); the two
additional conserved Cys residues found in LAM-1 are
indicated by (+). Of the multiple SCR present in each of
these proteins, the SCR with the highest homology to LAM-1
is diagrammed (Fig. 3C). A 15 amino acid spacer follows the
25   SCR units, preceding the transmembrane domain.

The expression of LAM-1 mRNA by cell lines of
lymphoid and non-lymphoid origin was examined. Northern
blot analysis revealed that LAM-1 cDNA hybridized strongly
to a 2.6 kb RNA species and weakly to a 1.7 kb RNA species
30   in poly(A)+ RNA isolated from the B cell lines Raji, SB,
Laz-509, and GK-5. However, RNA isolated from two pre-B
cell lines (Nalm-6, PB-697), three B cell lines (Namalwa,
Daudi, BJAB), five T cell lines (CEM, Hut-78, HSB-2, Molt-

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Cell samples and immunofluorescence analysis.

Mononuclear cells were isolated from human, rhesus monkey
(*Macaca mulatta*), cotton-topped tamarin (*Saguinus oedipus*),
dog (*Canis familiaris*), cat (*Felis catus*), sheep (*Ovis
aries*) or rabbit (*Oryctolagus cuniculus*) blood by Ficoll-
Hypaque density gradient centrifugation. Neutrophils were
isolated from blood samples at 20°C by centrifugation for
20 min at 1000 x g on a cushion of Mono-Poly Resolving
Medium (Flow Laboratories, McLean, VA) followed by lysis of
the red blood cells with ice-cold hypotonic 0.2% (w/v) NaCl
solution. Cells were obtained by protocols approved by the
Human Protection Committee and the Animal Care and Use
Committee of Dana-Farber Cancer Institute. Cells were kept
at 4°C and examined immediately after isolation.

Indirect immunofluorescence analysis was carried out
after washing the cells three times. The cells were then
incubated for 20 min on ice with each mAb as ascites fluid
diluted to the optimal concentration for immunostaining.
After washing, the cells were treated for 20 min at 4°C with
FITC-conjugated goat anti-mouse Ig antibodies (Tago,
Burlingame, CA). Single color immuno-fluorescence analysis
was performed on an Epics Profile flow cytometer (Coulter
Electronics, Hialeah, FL) or a FACStar (Becton Dickinson,
Mountain View, CA). Ten thousand cells were analyzed in
each instance.

Tissue sections were isolated from thymus and
mesenteric lymph nodes of rabbit, pig (*Sus scrofa*), goat
(*Capra hircus*), rat (*Rattus norvegicus*), guinea pig (*Cavia
porcellus*) and chicken (*Gallus domesticus*), and from rabbit
appendix. These frozen sections were stained with a given
anti-LAM-1 mAb at optimal concentrations, with subsequent
development using immunohistological procedures as described
(Mackay et al., J. Exp. Med. <u>167</u>:1755, 1988).

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LAM-1 mAbs as ascites fluid diluted at 1:100 in RPMI 1640
containing 5% fetal calf serum. The anti-LAM1-3 and -4 mAb
could be used at considerable dilution (1:5000) without a
decrease in their ability to inhibit HEV binding. The
5   cells, in a final volume of 100 μl, were then incubated
under rotation (64 rpm) for 30 min at 4°C on four 12 μm
frozen rat peripheral lymph node sections. After fixation
overnight in PBS with 1% (w/v) glutaraldehyde, the number of
HEVs per tissue section was determined and the number of
10   lymphocytes adherent to HEV was quantitated.

       Antibody cross-blocking experiments. Lymphocytes
(5 x 10^5) were first incubated with 10-fold saturating
concentrations of one anti-LAM-1 mAb as diluted ascites
fluid for 20 min on ice followed by the addition of optimal
15   concentrations of the second fluorochrome-labelled anti-
LAM-1 mAb to be used for direct immunofluorescence analysis.
After 20 min of further incubation, the cells were washed
and mAb binding assessed immediately by flow cytometry, as
described (Tedder et al., J. Immunol. 144:532, 1990).
20   Optimal concentrations for each antibody were determined by
indirect immunofluorescence analysis.

       **Domain mapping of epitopes defined by anti-LAM-1
mAb.** As described in detail elsewhere (Kansas et al., J.
Cell Biol. (in press) 1991), various domains of CD62 were
25   substituted for those of LAM-1 to create chimeric selectins
containing specific domains of LAM-1 with the remainder of
the protein as CD62. In particular, cDNAs encoding fusion
proteins containing the lectin domain, the lectin plus EGF
domains, or the lectin, EGF plus SCR domains of LAM-1, with
30   the remainder as CD62, were created and subcloned into the
Ap^{r}M8 expression vector (provided by Dr. Lloyd Klickstein,
Center for Blood Research, Boston, MA). These cDNAs were

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staining with the anti-LAM-1 mAb ranged from low staining
(+) to bright staining (++++) (Fig. 9, Table 7). No mAb
were detected that stained lymphocytes or neutrophils
preferentially. The level of staining observed was
5   characteristic of each mAb, and was not due to low or
insufficient mAb concentrations, because mAb levels greater
than saturation generated identical results; or to isotype
differences, because 14 of the 17 antibodies were of the
same Ig subclass (i.e. IgG_1).

10   **Inhibition of Lymphocyte binding to HEV is blocked
by some anti-LAM-1 mAb.** The anti-LAM-1 mAb were tested for
their ability to inhibit lymphocyte binding to HEV of rat
peripheral lymph node sections in the *in vitro* frozen
section assay (Stamper et al., J. Exp. Med. 144:828, 1976).
15   The anti-LAM1-3, anti-LAM1-4 and anti-LAM1-6 mAb inhibited
lymphocyte binding by 85 to 90% (Table 8). In contrast to
our previous report (Tedder et al., J. Immunol. 144:532,
1990), the anti-LAM1-1 and anti-LAM1-2 mAb consistently
inhibited binding to an intermediate degree (~65% and ~45%,
20   respectively). Our previous inability to detect this
inhibition may be secondary to the previous use of human
lymph nodes as a source of HEV; the likely involvement of
LFA-1 in binding to (presumably inflamed) human lymph nodes
(Stamper et al., J. Exp. Med. 144:828, 1976), may have
25   obscured the true effects of these mAb. The remaining 12
anti-LAM-1 mAb had little or no effect on lymphocyte binding
to HEV under the conditions examined (Table 7). This lack
of effect did not result from the presence of insufficient
mAb for inhibition, since each mAb was used at a
30   concentration 2 to 10 fold higher than that required for
saturation, as determined by immunofluorescence staining.

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LAM-1 can inhibit PPME binding while mAb binding to
different epitopes can upregulate binding.

    **Epitope localization of the anti-LAM-1 mAb binding
sites.** The spatial relationships between the different mAb
5   binding epitopes were examined by mAb cross-blocking
analysis (Table 9). Anti-LAM1-3 binding inhibited the
subsequent binding of anti-LAM1-4, anti-TQ1 and anti-LAM1-
2, but not anti-LAM1 mAb. Leu-8 binding was partially or
totally blocked by most of the other mAb with the exception
10 of anti-TQ1, anti-LAM1-2, -6 and -14. Taken together, this
suggests that the anti-LAM1-3 and -4 epitopes are close to,
but distinct from, the Leu-8 epitope, and farther from the
epitopes defined by the other mAb. Anti-LAM1-7 and -8
partially, and -9, -10, -11, -12 and -15, totally, inhibited
15 anti-LAM1-1, -5 and Leu-8 binding, indicating that these
epitopes are clustered closely together. Anti-LAM1-6
appeared unique in this analysis, and therefore defines a
spatially distant determinant. Finally, anti-LAM1-14
binding partially inhibited binding of anti-LAM1-1 only.
20 Therefore, while most mAb-defined epitopes appeared to be
closely associated within regions involved in ligand
binding, distinct mAb-binding epitopes could be
distinguished.

    **Domain mapping of epitopes identified by anti-LAM-1
25 mab.** To identify the structural domains that contain
epitopes defined by the anti-LAM-1 mAb, COS cells were
transfected with cDNAs encoding chimeric (LAM-1/CD62)
selectins and stained with anti-LAM-1 mAb (Table 10). With
the exception of anti-LAM-1, -5, -14 and -15, each mAb bound
30 the fusion protein containing only the lectin domain of LAM-
1, and hence recognized the lectin domain. In combination
with the mAb crossblocking analysis described above, this
indicates that multiple antigenic sites are present within

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detected (Table 11). The exceptions to this were the
epitopes identified by the anti-LAM1-8 and -9 mAb and the
epitopes identified by the anti-LAM1-10, -11 and -12 mAb,
which recognized LAM-1 from the same species.

5   The finding that human and mouse lymphocytes bind
PPME was extended to the study of tamarin, dog and rabbit
LAM-1$^+$ lymphocytes (Fig. 12). PPME binding to lymphocytes
from each of these species was completely inhibited by 5 mM
EDTA. In addition, preincubation of cells from each of

10   these three species with anti-LAM1-3 or -4 mAb diminished,
albeit incompletely, PPME-FITC binding (Fig. 12). Finally,
in two experiments, treatment of rhesus monkey blood
mononuclear cells with anti-LAM1-3 inhibited 53% and 88% of
lymphocyte binding to HEV, while treatment of dog

15   mononuclear cells with anti-LAM1-3 inhibited 52% of the HEV
binding. Thus, the functional and serologic properties of
LAM-1 appear to be well conserved in diverse and
phylogenetically disparate groups of mammals.

DISCUSSION

20   In this study, the LAM-1 epitopes which mediate or
regulate HEV- and PPME- binding were characterized using a
large panel of newly developed mAb (Table 7). In addition,
the physical proximites of the functional regions defined by
these mAb were identified, indicating that LAM-1 can be

25   divided into a number of overlapping regions associated with
distinct functional properties. Each mAb reacted with
leukocytes with characteristic levels of immunofluorescence
staining (Fig. 9, Table 7), suggesting that there may be
heterogeneity in expression of LAM-1 epitopes. Thus, many

30   of the anti-LAM-1 mAb may resemble the FMC46 mAb that
identifies a cell protrusion-associated epitope of LAM-1.
These mAb were also used to examine structural differences

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to bind both HEV and PPME. The DREG-56 mAb also fits into
this functionally defined group of mAb (Kishimoto et al.,.
Proc. Natl. Acad. Sci. USA 87:2244, 1990). The anti-LAM1-3
and -4 mAb bind the lectin domain of LAM-1 only (Table 10),
5   and each completely blocked binding of the other (Table 9).
The anti-LAM1-3 and anti-LAM1-4 mAb also completely block
neutrophil binding of PPME. Two additional mAb, anti-LAM1-
2 and TQ-1, also blocked PPME binding (Fig. 10). However,
these mAb were less efficient at blocking lymphocyte binding
10   to HEV (Table 8) suggesting that they may be more distant
from the ligand binding site than the LAM1-3 and -4
epitopes. These mAb collectively define one functional
group of LAM-1 epitopes and confirm previous work
demonstrating a close correlation between the lectin
15   activity of LAM-1 (and MEL-14) and the HEV binding ability
of leukocytes (Yednock et al., J. Cell. Biol. 104713, 1987;
Yednock et al., J. Cell. Biol. 104:725, 1987; Stoolman et
al., J. Cell. Biol. 99:1535, 1984; Rosen et al., J. Immunol.
142:1895, 1989; Stoolman et al., J. Clin. Invest. 84:1196,
20   1989; Stoolman et al., Blood 70:1842, 1987).

     Activation of lymphocytes through CD2 or CD3, or
activation of neutrophils with TNF-α or GM-CSF, induces a
rapid and transient increase in the affinity of LAM-1 for
its ligand. An interesting and unexpected result of the
25   current studies was that binding of the anti-LAM1-1 and -5
mAb resulted in an identical increase in PPME binding by
LAM-1 (Fig. 10). Crossblocking studies indicated that these
mAb bind to the same or overlapping regions, distinct from
those defined by the anti-LAM1-3 and -4 mAb. Interestingly,
30   domain mapping studies demonstrated that these epitopes are
in, or require the presence of, the LAM-1 EGF-like domain.
However, only the anti-LAM1-1 mAb inhibited lymphocyte
binding to HEV. The anti-LAM1-15 mAb, which blocks the

suggesting that the ligand for both murine and human
lymphocytes is well conserved through recent mammalian
evolution (Stoolman et al., Blood 70:1842, 1987; Stamper et
al., J. Exp. Med. 144:828, 1976; Butcher et al., J. Immunol.
5   134:2989, 1979; Butcher et al., Nature 280:496, 1979; Wu et
al., J. Cell. Biol. 107:1845). The demonstration that dog,
tamarin and rabbit lymphocytes bind PPME in a calcium-
dependent fashion (Fig. 12), in combination with the
observation that dog and rhesus monkey lymphocytes utilized
10   LAM-1 to bind to rodent HEV, confirms and extends this
observation. These findings also indicate that the homing
receptor expressed on leukocytes from these species involves
a C-type lectin activity (Drickamer, J. Biol. Chem.
263:9557, 1988). Consistent with this, each mammal species
15   (excluding rodents) tested expressed one or more of the LAM-
1 epitopes (Table 11). The LAM1-3 epitope was the most
broadly conserved among the animal species examined, further
suggesting a critical function for this region in leukocyte
migration. The reactivity of each mAb with numerous animal
20   species also indicated that while several mAb were reactive
with functionally identical regions of human LAM-1, most mAb
identified unique epitopes. It is likely that subtle amino
acid changes in LAM-1 between species accounts for the
differences between reactivities of the anti-LAM-1 mAb.
25   Thus these findings provide a mechanistic explanation for
the conservation of receptor function, in that the regions
of the molecule critical for adhesion were best conserved.
Obtaining the primary amino acid sequences from LAM-1 cDNAs
isolated from each species in the future will also be
30   informative, but interpretation of sequence information in
regards to ligand binding sites and functional epitopes of
the molecule will require the exact knowledge of the three
dimensional structure of LAM-1.

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on lymphocytes is 74,000 and that f neutrophils 1 90,000-100,000 [6,9,12].

The specific adhesion of some tumor cells to the capillary endothelium and the existence of organ specific metastasis suggest that interactions between tumor cells and normal tissues influence tumor localization [13-15]. Although the molecules that mediate these events in malignant cells have not been completely described, many cell surface molecules involved in the adhesion and migration of normal leukocytes may be involved in the dissemination of hematopoietic malignant cells [1-3]. The mLHR has been implicated in the dissemination of lymphomas [14-16], and a calcium-dependent phosphomannosyl-binding site on human malignant lymphoblastoid cell lines mediates peripheral LN HEV binding [17]. In the invention described herein, the structure, function and regulation of LAM-1 expression was examined on normal lymphocytes and compared to LAM-1 of malignant leukocytes.

The LAM-1 molecule is a member of a new family of cellular adhesion/homing molecules that contain a lectin-like domain at their amino-terminal end followed by an epidermal growth factor-like domain and short consensus repeat units like those found in C3/C4 binding proteins. In J. Exp. Med., 170: 123-133 (1989) [4] and co-pending Application Serial No. 07/313,109, T.F. Tedder et al. report the isolation and chromosomal localization of cDNAs encoding the novel cell surfac mol cul LAM-1. In Eur. J. Immun l., 20: 1351-1355 (1990), T.F. Tedd r t al. reported that human antigen-sp cific memory T c lls xpress th LAM-1 necessary for lymphocyt recirculation. In J. Bi logical Chemistry,

plays a key role in determining the characteristi s and mag-
nitude of local immune responses (5).

The present invention relates to the production of a
new antibody to LAM-1. The new monoclonal antibody,

5   anti-LAM1-3, is useful in radioisotope or immunofluorescent
assays for the detection of LAM-1. For example, identifying
species which have or do not have LAM-1. The antibody is
further useful for separating cells expressing LAM-1 from
cells not expressing LAM-1 or visa versa. Furthermore, this

10   monoclonal antibody also completely blocks leukocyte at-
tachment to HEV or endothelium.

Neutrophil-mediated inflammation is involved in a num-
ber of human clinical manifestations, including the adult
respiratory distress syndrome, multi-organ failure and

15   reperfusion injury. One way of inhibiting this type of
inflammatory response would be to block competitively the
adhesive interactions between neutrophils and the endo-
thelium adjacent to the inflamed region. Anti-LAM1-3 reacts
with LAM-1 on many animal species, but does not bind the

20   mLHR. Anti-LAM1-3 blocks completely lymphocytic traffic to
lymph nodes and extravasation of neutrophils from blood to
inflammatory sites. The administration of soluble forms of
anti-LAM1-3 could be clinically effective for the inhibition
of neutrophil-mediated inflammation. Anti-LAM1-3 also

25   blocks lymphocyte adhesion to human HEV and activated endo-
thelium. Therefore, it is likely that the use of anti-LAM1-
3 will block lymphocyte entry into sites of inflammation or
tissue injury. Such activity will be useful for pr v nting
kidn y or other organ transplant rej ction which is m diat d

30   by lymphocytes.

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STATEMENT OF DEPOSIT

A hybrid cell line which produces the anti-LAM-1 mon -
clonal antibody anti-LAM1-3 embodying this invention was
been deposited with the American Type Culture Collection,
12301 Parklawn Drive, Rockville, Maryland 20852 on June 12,
5   1991 and is assigned A.T.C.C. Deposit No. HB 10771.

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protein (GMP-140, PADGEM, CD62) which is expressed
on activated platelets and endothelial cells, and
the human endothelial leukocyte adhesion molecule-
1 (ELAM-1) expressed on activated endothelial

5   cells. The name "selectins" has been suggested
for this family because of the presence of the
lectin domain and their role in selective cell
trafficking.

LN   = lymph node

10   PMA   = phorbol 12-myristate 13-acetate

PKC   = protein kinease C

LAM-1   = leukocyte adhesion molecule-1

CLL   = chronic lymphocytic leukemia

NHL   = non-Hodgkin's lymphoma

15   PPME   = poly-phosphomonoester from the yeast HANSENULA
cell wall

AML   = adult myelogenous leukemia

CML   = chronic myelogenous leukemia

PBMC   = peripheral blood mononuclear cells

20   BM   = bone marrow

RPMI Medium = commercial product available from Gibco,
Walkersville, Maryland.

FSC   = follicular small cleaved cell lymphoma

CSF   = colony stimulating factor

25   DSC   = diffuse small cleaved cell lymphoma

FITC   = fluorescein isothiocyanate

LPS   = lipopolysaccharide

kb   = kilobase

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shedding is similar to that of mLHR (33-34). This is con-
sistant with prior observations that the cell surface
expression of LAM-1 is rapidly down-regulated upon ac-
tivation (5,6,11). LAM-1 removal from the cell surface may
5   thus be necessary for the detachment of leukocytes from the
endothelium so as to allow for their subsequent migration
into tissues.

LAM-1 was most frequently expressed by CLL cells among
the various hematologic malignancies studied (Table 1,
10   Fig. 5). These results extend previous studies of LAM-1
expression to TQ1 and Leu-8 using CLL and NHL cells [35-37].
Since the expression of LAM-1 was somewhat restricted among
hematologic malignancies, the expression, or absence of
expression, may have a major impact on the trafficking of
15   leukemic cells and the dissemination of NHL. Immuno-
precipitation of LAM-1 from CLL cells showed that it
resembled the Mr 74,000 isoform of the glycoprotein
expressed by normal lymphocytes (Fig. 6). In addition,
LAM-1 expressed by malignant cells was functional since
20   LAM-1 on normal lymphocytes and CLL cells were both able to
bind HEV and PPME (Table 2, Fig. 7). Both HEV and PPME
binding was mediated by LAM-1 since the new monoclonal an-
tibody, anti-LAM1-3, was able to completely block all HEV
and PPME binding.

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research group using lymphoblastoid cell lines [17]. CD44
constitutes a broadly distributed family of glycoproteins
expressed on virtually all hematopoietic cells, fibroblasts,
epidermal, glial and melanocytic origin cells [21,38]. Al-
5   though CD44 was initially regarded as the human homing
receptor equivalent of the mLHR [28,29], it may be more gen-
erally involved in cell-cell or cell-matrix binding as a
receptor for hyaluronate [39]. Previous studies have also
suggested that CD44 is involved in the dissemination of NHL
10 [40]. During the work resulting in the present invention,
however, no clear relationship could be inferred from the
results of CD44 expression alone.

LAM-1 is expressed on most neutrophils, monocytes, nor-
mal myeloid progenitor cells and early erythroid precursors
15 in BM (bone marrow) [6]. The co-expression of this homing
receptor and other adhesion molecules may control the
physiological retention (homing) of these cells in BM. The
homing of intravenously transplanted hematopoietic stem
cells is mediated by a recognition system with galactosyl
20 and mannosyl specificities [41] which might also mimic the
LAM-1 ligand [42]. In this regard, it is noted that AML and
CML cells were found to lack expression of LAM-1. Unlike
the situation with lymphoid tumors, this is in sharp dis-
tinction with the high level expression of LAM-1 on normal
25 myeloid cells. The absence of LAM-1 expression on most AML
and CML cells might favor the passage of these cells into
the bloodstream. Although overnight culture of CML cells
did not result in the expression of LAM-1 on th c ll sur-
fac , the overall lack of LAM-1 expr ssion by these c lls
30 indicat s that further investigations of the regulation of
LAM-1 by l ukemic myeloid cells is warranted.

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th Human Protection Committee f the Dana-Farb r Cancer

Institute. Tumor type was classified according to conven-

tional morphological, cytological and immunophenotype

criteria. Tumor cell lineage was determined by analysis of

5   antigens (Ag) including surface and cytoplasmic immuno-

globulin (Ig), HLA-DR Ag, CD1, CD2, CD3, CD4, CD5, CD6, CD8,

CD9, CD10, CD11b, CD13, CD14, CD19, CD20 and CD33. Cells

were examined immediately after isolation or were immediate-

ly cryopreserved and kept frozen in liquid nitrogen until

10   used. The frequency of malignant cells was always greater

than 90% in every sample examined.

Antibodies.

        The anti-LAM-1 monoclonal antibodies anti-LAM1-1

and anti-LAM1-2 and the monoclonal antibody anti-TQ1 have

15   been previously described [5,8]. The anti-LAM1-3 antibody

(IgG1) of the claimed invention was generated by the fusion

of NS-1 myeloma cells with spleen cells from Balb/c mice

that were repeatedly immunized with cells of the mouse pre-B

cell line 300.19 transfected with a LAM-1 cDNA as described

20   [5]. The antibodies used in these studies included: 2F12

(CD11a) and 10F12 (CD18) [18] which were gifts from J. Ritz

(Dana-Farber Cancer Inst., Boston MA); TS2/9 (CD58, anti-

LFA-3) [19] and RR 1/1 (CD54, anti-ICAM-1) [20] which were

gifts from T.A. Springer (Center for Blood Research, Boston,

25   MA); 515 (CD44) [21] a gift from G.S. Kansas (Dana-Farber

Cancer Inst.); and 904 (CD11b) [22].

Immunofluorescence analysis.

        Indirect immunofluoresc nce analysis was p rform d

on viabl cells isolated by Ficoll-Hypaque density gradient

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Immunopre ipitati n analysis

          Cells were washed twice, resuspended in RPMI 1640

medium (Sigma, St. Louis, MO) at a concentration of 30 x 10^6

cells/ml) and treated for 40 minutes at room temperature

with neuraminidase (0.1 U/ml, Calbiochem, La Jolla, CA) and

5   then labelled by lactoperoxidase-catalyzed iodination.

After washing, the cells were lysed in buffer containing 1%

(v/v) NP-40 as described [27]. Cell lysates were precleared

for 2 hours using 3 µl of murine ascites fluid (isotype

matched antibody) and 25 µl of a 50% (v/v) suspension of

10   Gammabind-G Agarose (Genex, Gaithersburg, MD). Cell lysates

were precleared again overnight. Half of the precleared

lysate was then incubated with 3 µl of anti-TQ1 ascites

fluid, 3 µl of anti-LAM1-1 ascites fluid, and 50 µl of

Gammabind-G with constant rotation at 4°C for 18 hours. The

15   other half of the lysate was treated similarly using 3 µl of

isotype-matched murine ascites fluid. Immunoprecipitates

were washed and analyzed by SDS-PAGE. Molecular weights

(Mr) were determined using standard molecular weight markers

(BRL, Bethesda, MD).

20   In experiments designed to study LAM-1 shedding, LAM-1

was immunoprecipitated as described above from the super-

natant fluid and the pellet of PBMC that had been cultured

for 60 minutes at 37°C in RPMI 1640 medium alone or in RPMI

medium containing PMA (100 ng/ml, Sigma, St. Louis, MO). In

25   addition, expression of LAM-1 was assessed after incubation

of th c lls with PMA (10 nM for 30 minutes) following the

prior culture of the cells with sodium azide (Sigma) or th

protein kinas inhibitors, 1-(5-Isoquinolinyl-sulfonyl)-2-

methylpiperazine (H-7, Calbiochem) and staurosporine (Sigma)

30   for 30 minutes at 37°C.

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myelog nous l ukemia; CML, chr nic myelogenou leukemia).
The relative fluorescence staining intensity of the malig-
nant cells is indicated where the positive population could
be identified as a distinguishable peak from background flu-
orescence staining: ±, where a shoulder of positively
stained cells was evident, +, where a separate peak of posi-
tive cells was identified with weak fluorescence; ++, a
definite separate peak of fluorescence positive cells of
moderate fluorescence; +++, a peak of fluorescence positive
cells of the same intensity as normal blood lymphocytes.
The tissue source of all malignant cells is also indicated.

Figure 6.

Anaylsis of LAM-1 immunoprecipitated from B-CLL
cells. Detergent lysates of surface iodinated cells (45 x
10^6) were immunoprecipitated with the anti-TQ1 and anti-
LAM1-1 antibodies (LAM-1) or an unreactive isotype-matched
antibody control (Cont.). Immunoprecipitated materials wer
divided and analyzed under non-reducing and reducing con-
ditions on a 12% SDS polyacrylamide gel followed by
autoradiography. Molecular weights (kDa) were determined by
the migration of known protein standards.

Figure 7.

Normal human lymphocytes and CLL cells are capable
of binding PPME through LAM-1. Cells were examined for
LAM-1 expression by indirect immunofluorescence analysis
after treatment with the anti-LAM1-3 monoclonal antibody
(dark line) or with an unreactiv isotype matched antibody
(thin line). Cells were also reacted with FITC-conjugated
PPME aft r treatment with th anti-LAM1-3 antibody (thin
line) or an unreactive control antibody (dark lin). The

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iodinati n sinc LAM-1 may be mor readily immuno-
precipitated after the removal of sialic acid residues.
Treatment of cells with PMA resulted in a dramatic loss of
immunoprecipitable LAM-1 from the cell surface with a con-
5   comitant increase in the level of LAM-1 precipitated from
the supernatant fluid. Incubation of cells in medium with-
out PMA also resulted in a small amount of LAM-1 being found
in the supernatant fluid (Fig. 4). The molecular weight of
LAM-1 precipitated from the supernatant fluid was slightly
10   smaller (by about 5 kDa) than the species of LAM-1 found on
the cell surface. Interestingly, the residual LAM-1 found
on the cell surface of PMA-treated cells was most similar in
molecular weight to that of the LAM-1 found in the super-
natant fluid. The quantititive recovery of labeled LAM-1
15   from the supernatant fluid, in comparison to the amount
immunoprecipitated from solubilized cells, demonstrates that
a major portion of LAM-1 is shed from the cell surface and
not internalized following PMA exposure.

       Expression of adhesion molecules by malignant
20   leukocytes.
              The expression of LAM-1 and other cell surface
molecules known to be involved in lymphocyte adhesion and
migration was examined on malignant leukocytes from 118
patients by indirect immunofluorescence analysis. LAM-1
25   expression was most frequently demonstrated on CLL cells and
among lymphomas classified as follicular (FSC) and diffuse
small cleav d cell lymphoma (DSC) (Table 3). On the other
hand, most acute myeloblastic leuk mia (AML), acute lympho-
blastic leukemia (ALL), chronic my locytic leukemia (CML),

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appearanc of. LAM-1 on the ell surfaces or a significant

increase in the frequency of LAM-1 expression in any case.

Structure of LAM-1 expressed by malignant cells.

Anti-LAM-1 antibodies were used to immuno-

5   precipitate LAM-1 from CLL cells. LAM-1 migrated with a Mr

of 68,000 under non-reducing conditions and at 73,000 after

reduction (FIG. 6), similar to LAM-1 immunoprecipitated from

normal lymphocytes (FIG. 4). Therefore, it appears that

normal and malignant lymphocytes express the same cell-

10   surface LAM-1 protein.

LAM-1 receptor function.

The relationship between LAM-1 expression and the

ability of cells to bind to human peripheral LN HEV was

examined using cells from normal circulating blood, three

15   LAM-1 positive CLLs and one LAM-1 negative CLL. Cells were

assessed for their ability to bind HEV of human peripheral

LN using the frozen section assay of Stamper and Woodruff

[25]. The LAM-1+ cells bound to HEV at levels which corres-

ponded to the amount of LAM-1 expressed on their cell sur-

20   face, while the LAM-1 CLL cells did not bind (Table 2). In

contrast, CD44 expression was quite high on all of the cell

samples examined and did not correlate with HEV adhesion.

Additional studies examined the ability of anti-LAM-1 monoc-

lonal antibody to block HEV binding. A new antibody, anti-

25   LAM1-3, was abl to sp cifically block 92 to 95% of normal

lymphocyt and LAM-1+ CLL cell binding (cells from Table 2)

to rat p ripheral LN HEV. In contrast, the binding f a

different antibody, anti-LAM1-10, reactive with a différent

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LAM-1+ CLL cell samples test d furth r. PMA xposure le d to
the complete loss of LAM-1 expression after 180 to 360
minutes of culture with PMA with similar kinetics to those
of RAJI cells treated simultaneously. These experiments
were also carried out using cryo-preserved blood lymphocytes
and RAJI cells as controls, with no appreciable affect on
the ability of the cells to modulate LAM-1 expression after
PMA exposure.

The down-modulation of LAM-1 expression was also inves-
tigated in LAM-1 cDNA transfected cells. The erythro-
leukemia cell line, K562, and the mouse pre-B cell line,
300.19, were transfected with LAM-1 cDNA as described [5],
generating cells that express relatively high levels of cell
surface LAM-1 (Fig. 8). In contrast to what was observed
with RAJI cells and the majority of CLL cells, 90 minutes
exposure of these cells to PMA induced an almost complete
loss of LAM-1 from the cell surface.

The role of PKC in LAM-1 shedding was further assessed
by culturing normal blood lymphocytes with protein kinase
prior to their exposure to PMA. Treatment of cells with
both H-7 [31] and staurosporine [32] inhibited shedding, al-
beit at different optimal molar concentrations (Table 1).
In contrast, pretreatment of lymphocytes with sodium azide
did not inhibit down-modulation of cell surface receptor.
However, the shedding process required on-going metabolism
since PMA treatment at 4°C did not induce detectable LAM-1
sh dding (data not shown). Thus, PKC may r gulat cell sur-
fac r ceptor expression through dir ct phosphorylati n of
LAM-1 which may signal for cleavag or through kinase
reguation of prot ase activity.

two patients with AML, one with ALL and on with CML) w re
incubated overnight at 37°C in RPMI 1640 medium containing
10% FCS. Contrary to the results obtained. under the same
conditions with normal spleen cells [5], an increase in

5   LAM-1 expression was not detected on the malignant cells
after culture, suggesting that LAM-1 was not expressed
constitutively on these cells.

Table 5, below, lists a number of the properties of the
monoclonal antibodies anti-LAM1-1, -2 and -3. Lines 1, 2,

10   and 3 give the name of the antibody, the isotype and the
differences in staining intensity. These properties are not
necesarily indicative of differences in epitope recognition.

Functional studies of the antibodies are given in lines
4-6. These results demonstrate that different parts of the

15   LAM-1 molecule are recognized by the different monoclonal
antibodies. Lymph nodes contain structures called high en-
dothelial venules (HEV) which are utilized by lymphocytes to
enter the lymph nodes (the site of immune responses) from
the blood stream. Emigration of lymphocytes into the node

20   has been shown to be mediated by adhesion molecules which
allow the cells to stick to and then traverse the venule.
This process has been studied by incubating isolated
lymphocytes with lymph node tissue sections. When the sec-
tions are incubated with lymphocytes alone, the cells will

25   adhere to HEV, and the number of adherent cells can be
counted. Various monoclonal antibodies, including the LAM-1
antibodies, have be n used to block this binding, Lin 4
giv s the results of such studies for LAM1-1, -2 and -3.

The polysaccharide PPME mimics the natural ligand for

30   the LAM-1 mol cul . Since PPME can b directly fluores-

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LAM1-3 strongly blocks it. Speci s cross-reactivity giv s
further indications of the diferences which exist between
the antibodies and the epitopes that they identify.

Line 11 gives the results of the domain mapping regard-
ing the monoclonal antibodies. The LAM-1 molecule contains
three domains which are:

(a) a lectin-like domain (L);

(b) an epidermal growth factor-like domain (EGF); and

(c) a domain of short consesus repeats (SCR).

In order to determine which domain was recognized by each
antibody, cDNAs were constructed which contained the infor-
mation coding for:

(1) the whole LAM-1 molecule;

(2) the L, EGF and SCR domains from the LAM-1 molecule;

(3) the L domain from the LAM-1 plus EGF and SCR
    domains from the CD62 molecule of the same family
    of proteins;

(4) the L plus EGF domains from LAM-1 (SCR from
    CD62; and

(5) the L plus SCR domains from LAM-1 (EGF from CD62).

These cDNAs were transfected into cells which then produced
the corresponding proteins. The pattern of reactivity of
the various monoclonal antibodies was then determined as
shown in Table 6, and the domain necessary for monoclonal
antibody reactivity was assigned. For example, anti-LAM1-3
bound to cells expressing all the domains described with
m dium to very strong strength. Anti-LAM1-1, however, did
not bind to c lls which contained LAM-1 (L + SCR) or LAM-1
(L) alone. The pitope which is recognized by anti-LAM1-1
must, ther fore, be compos d of a sit within the EGF,

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The wide conservation of the LAM-1 epitopes
expressed throughout recent mammalian evolution, in
combination with the functional studies demonstrating a
molecular component similar to LAM-1 in function and
5   specificity, underscores the critical role of LAM-1 in the
regulation of leukocyte migration in multiple animal
species. Thus, LAM-1 may be the most functionally and
structurally conserved leukocyte adhesion molecule found in
mammals, considering that many do not function reciprocally
10   between man and mouse. Whether the evolutionary basis for
this high level of conservation is fundamental to the
function of the receptor or results from the unique nature
of a carbohydrate-based ligand with limited potential for
divergence will have to be determined after identification
15   and characterization of the true ligand(s).

Use
As leukocyte migration and infiltration into areas
of tissue damage or injury or tissue transplant can cause or
increase pathology, agents that impede these processes can
20   be used for therapeutic treatment. Specifically, leukocyte-
mediated inflammation is involved in a number of human
clinical manifestations, including the adult respiratory
distress syndrome, multi-organ failure and reperfusion
injury. One way of inhibiting this type of inflammatory
25   response would be to block competitively the adhesive
interactions between leukocytes and the endothelium adjacent
to the inflamed region. As LAM-1 mediates the migration and
adhesion of blood leukocytes, treatment of a patient in
shock, e.g., from a serious injury, with an antagonist to
30   c ll surface LAM-1 function (such as the monoclonal
antibodies of the invention) can result in the reduction of

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Table 1.   LAM-1 Shedding Is Blocked By Protein
            Kinease Inhibitors

	Without PMA[a] Treatment		With PMA Treatment	
Cells with	% Positive	MCF No.	% Positive	MCF No.
Medium	60	115	13	47
Stauro				
porine 1mM	57	91	54	95
500µM	72	95	49	91
100µM	51	88	51	92
50µM	47	92	58	100
H-7 1mM	52	94	53	92
500µM	67	102	32	80
100µM	58	109	23	91
50µM	61	106	11	61
NaN$_3$ 1µM	59	113	20	50

a =   The percentage of cells reactive with the anti-
       LAM1-1 monoclonal antibody was determined by in-
       direct immunofluorescence analysis. The relative
       intensity of staining of the positive cells is in-
       dicated based on the mean fluorescence channel
       number (MFC No.) obtained with FACS analysis (256
       channels, on a 3-decade log scale. C lls treat d
       with an unreactiv monoclonal antibody had 3% pos-
       itiv cells with a MFC no. of 40.

Table 3

EXPRESSION OF ADHESION MOLECULES BY MALIGNANT LEUKOCYTES

Diagnosis	No. of cases expressing antigen / number examined[a] (mean % among positive cases)						
	LAM-1	CD44	CD11a	CD11b	CD18	CD54	CD58
Pre-B-ALL							
CD10+	4/15 (32)	10/12 (67)	1/8 (46)	0/15	1/12 (40)	4/13 (33)	6/13 (69)
CD10-	0/8	5/5 (80)	0/6	0/5	0/5	0/5	2/5 (55)
T-ALL	2/10 (31)	8/9 (67)	3/8 (59)	0/2	5/10 (49)	4/10 (46)	1/10 (62)
B-CLL	16/27 (55)	16/18 (76)	3/14 (45)	0/21	3/18 (32)	1/18 (31)	1/18 (43)
B-lymphoma							
FSC	6/12 (32)	9/11 (59)	8/9 (58)	0/12	9/11 (38)	5/11 (50)	2/11 (49)
DSC	2/4 (41)	4/4 (70)	1/4 (90)	0/3	1/4 (90)	3/4 (40)	1/4 (77)
DLC	1/6 (27)	6/6 (59)	5/6 (55)	0/6	4/4 (49)	3/6 (54)	2/6 (53)
Burkitt's	0/4	2/4 (52)	0/4	0/4	0/4	1/4 (72)	0/4
M.Myeloma	0/3	2/2 (84)	3/3 (53)		2/2 (37)	0/3	1/3 (52)
AML	2/19 (44)	16/16 (79)	6/11 (50)	5/19 (42)	11/16 (41)	1/16 (47)	9/18 (61)
CML	0/12	10/10 (61)	5/10 (43)	2/12 (48)	5/10 (39)	0/10	4/10 (70)

[a]Cases were considered positive for the Ag being examined if > 25% of the cells were positive.

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2.   HEV binding was assessed using 12-μm freshly cut,
frozen rat lymph node sections. The number of
lymphocytes bound to HEV was counted on coded slides.
Values are means ± standard deviation (s.d.) of four
5   experiments, and the differences between control
antibody-treated cells and anti-CD2 and anti-CD3
treated cells were significant.

3.   P<0.05 using the paired Student's t-test.

4.   P<0.01 using the paired Student's t-test

1 = Flu r   nc int nsity, human lymphocyte and peripheral
    mononuclear cells. Fluorescence intensity of staining
    in indirect immunofluorescence assays, is given on a
    4-(+) scale where (-) indicates no specific reactivity
    and (++++) indicates the highest level of activity.

2 = Monoclonal antibody blocking, High Endothelial
    Venule binding.

3 = Monoclonal antibody blocking, phosphomannan monoester
    fragments (PPME).

4 = PPME blocking monoclonal antibody.

5 = Ability to block binding of antibody.

6 = Domain mapped.

Abbreviations

Bind = Binding

B = Blocks

wB = weakly Blocks

E = Enhances

ND = Not Done

L = Lectin

EGF = Epidermal Growth Factor-like

SCR = Short Consensus Repeats

N = No effect

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Table 7

Properties of the anti-LAM-1 mAb used in this study

Name	mAb Isotype	Staining Intensity[a]		HEV[b] Binding	Effect[c] on PPME
		Lympho.	Neutro.		
TQ1	G1	++	++	N	B
LAM1-1	G1	+++	+++	B	E
LAM1-2	M	++	++	wB	B
LAM1-3	G1	++++	++++	B	B
LAM1-4	G1	+++	+++	B	B
LAM1-5	G1	+++	+++	N	E
LAM1-6	G1	+	+	B	N
LAM1-7	G1	++	++	N	N
LAM1-8	G1	+++	+++	N	N
LAM1-9	G1	+++	+++	N	N
LAM1-10	G1	+++	+++	N	N
LAM1-11	G1	+++	+++	N	N
LAM1-12	G1	+++	+++	N	N
LAM1-13	M	+	+	N	N
LAM1-14	G1	+++	++	N	N
LAM1-15	G1	++	++	N	N
LAM1-16	M	+	+	N	N

[a] The reactivity of lymphocytes and neutrophils with the anti-LAM-1 mAb were determined by indirect immunofluorescence analysis with the relative intensity of fluorescence staining indicated on a - (no reactivity) to ++++ (highest reactivity) scale as in Fig. 1. Results are representative of those obtained in at least three experiments.

[b] The ability of each mAb to inhibit lymphocyte binding to rat HEV was assessed as in Table 8. B = Blocks binding, E = Enhances binding, N = No significant or detectable effect on binding, w = weak effect. Results are representative of those obtained in at least two xperiments.

[c] The ability of each mAb to inhibit PPME binding to human lymphocytes was as in Fig. 2. Results are representative of those obtained in at least two experiments.

Table 9

Cross-Blocking Experiments with Anti-LAM-1 mAb[a]

Test mAb:	Ability of the test mAb to block the binding of labeled:				
	anti-TQ1	Leu-8	LAM1-1	LAM1-3	LAM1-5
TQ1	+++	-	-	+++	ND
LAM1-1	-	++	+++	-	+++
LAM1-2	+++	-	-	+++	ND
LAM1-3	+++	+++	-	+++	-
LAM1-4	+++	+++	-	+++	-
LAM1-5	++	++	+++	-	+++
LAM1-6	+++	-	+	-	-
LAM1-7	+++	++	++	-	++
LAM1-8	-	++	++	-	++
LAM1-9	-	+++	+++	-	+++
LAM1-10	-	+++	+++	-	+++
LAM1-11	-	+++	+++	-	+++
LAM1-12	-	+++	+++	-	+++
LAM1-14	-	-	++	-	-
LAM1-15	-	++	+++	-	+++

a Values represent the relative ability of the test mAb to block the binding of the indicated FITC-labeled antibodies: -, did not inhibit; +, partial inhibition; ++, significant but incomplete inhibition; +++, complete inhibition of mAb binding. These results are representative of those obtained in three experiments.

Table 11

CONSERVATION OF LAM-1 EPITOPES AMONG DIFFERENT ANIMAL SPECIES

mAb[b]	HUMAN[a]	RHESUS[a]	TAMARIN[a]	COW[a]	RABBIT[a,b]	SHEEP[a]	DOG[a]	CAT[a]	PIG[b]	GOAT[a]	RAT[a,b]	GUINEA PIG[b]	CHICKEN[b]
TQ1	+++	-	-	ND	+++	-	-	-	-	-	ND	ND	ND
LAM1-1	+++	+++	+++	ND	ND	-	-	-	ND	ND	ND	ND	ND
LAM1-2	+++	-	++	ND	++	-	-	-	ND	ND	ND	ND	ND
LAM1-3	++++	+	-	+++	++++	+++	+++	-	-	+++	-	-	-
LAM1-4	+++	-	++	+++	++	-	-	-	-	ND	-	-	-
LAM1-5	+++	+++	+++	+++	+	+++	-	-	-	-	-	-	-
LAM1-6	+++	+	+	-	+	-	-	-	-	-	-	-	-
LAM1-7	+++	++	++	++	++	++	-	-	-	-	-	-	-
LAM1-8	+++	++	++	+++	+	++	-	-	-	-	-	-	-
LAM1-9	+++	++	++	+++	-	+++	-	-	-	-	-	-	-
LAM1-10	+++	++	++	+++	-	-	-	-	-	-	-	-	-
LAM1-11	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-
LAM1-12	+++	+++	++	++	-	ND	-	-	-	-	-	-	-
LAM1-13	±	-	-	-	+	+	-	-	-	-	-	-	-
LAM1-14	+++	+	+	++	++	-	-	+	-	ND	-	-	-
LAM1-15	+++	++	++	+++	-	+++	-	-	-	++	-	-	-
LAM1-16	+	-	+	-	-	+	-	-	-	ND	-	+	-

a Reactivity and relative fluorescence intensity of staining was determined by flow cytometry analysis of blood leukocytes. The scale of fluorescence intensities is as in Fig. 1 with - indicating no significant reactivity. ND, not determined.

b Reactivity of staining was determined by immunohistology of thymus, lymph nodes and rabbit appendix.

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leukocyte migration to a level manageable by the target
endothelial cells.

In addition, agents developed to block receptor
function can inhibit the metastasis and homing of malignant
5   cells which express the LAM-1 receptor protein.

The therapeutic agents may be administered orally,
parenterally, or topically by routine methods in
pharmaceutically acceptable inert carrier substances.
Optimal dosage and modes of administration can readily be
10 determined by conventional protocols.

The normal regulation of the *lyam-1* gene (the name
given to the human gene encoding LAM-1), as evidenced by the
appearance and disappearance of the LAM-1 protein on the
surface of a specific leukocyte subpopulation, can be
15 monitored by use of the monoclonal antibodies of the
invention, in order to test the effects of drugs or specific
therapies that may alter gene expression.

What is claimed is:

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1   7.   The method of claim 5, wherein said monoclonal
2 antibody is anti-LAM1-4 monoclonal antibody.

1   8.   A method of detecting leukocyte activation in an
2 animal, said method comprising
3   obtaining a fluid sample from said animal;
4   contacting said sample with the monoclonal antibody
5 of claim 1; and
6   detecting formation of immune complexes formed by
7 said monoclonal antibody, said immune complexes indicating
8 the presence of LAM-1 shed from activated leukocytes.

1   9.   The method of claim 8, wherein said monoclonal
2 antibody is anti-LAM1-4 monoclonal antibody.

1   10. A method of blocking leukocyte interactions
2 with endothelium in an animal, said method comprising
3 administering an effective amount of the monoclonal antibody
4 or fragment thereof of claim 1 to said animal.

1   11. The method of claim 10, wherein said monoclonal
2 antibody is anti-LAM1-4 monoclonal antibody.

1   12. The method of claim 10, wherein said animal is
2 a human.

1   13. The method of claim 10, wherein said animal is
2 suffering from inflammation.

1   14. The method of claim 10, wherein said animal is
2 suffering from an autoimmune response.

5   contacting said cells with a reagent comprising the
6   monoclonal antibody of claim 18; and
7   determining which cells form an immune complex with
8   said reagent.

1   23. A method of isolating cells expressing LAM-1,
2   said method comprising
3   providing a sample of cells, at least some of which
4   are suspected of expressing LAM-1;
5   contacting said cells with the monoclonal antibody
6   of claim 18; and
7   separating those cells which have formed an immune
8   complex with said monoclonal antibody, from those cells
9   which have not.

1   24. The method of claim 22, wherein said monoclonal
2   antibody is anti-LAM1-5 monoclonal antibody.

1   25. A method of detecting leukocyte activation in
2   an animal, said method comprising
3   obtaining a fluid sample from said animal;
4   contacting said sample with the monoclonal antibody
5   of claim 18; and
6   detecting formation of immune complexes formed by
7   said monoclonal antibody, said immune complexes indicating
8   the presence of LAM-1 shed from activated leukocytes.

1   26. The method of claim 25, wherein said monoclonal
2   antibody is anti-LAM1-5 monoclonal antibody.

1   27. A method of blocking leukocyte interactions
2   with endothelium in an animal, said method comprising

1   35.   A monoclonal antibody which recognizes the LAM-
2   1 epitope recognized by anti-LAM1-6 monoclonal antibody.

1   36.   The monoclonal antibody of claim 35, wherein
2   said monoclonal antibody is anti-LAM1-6 monoclonal antibody.

1   37.   A hybridoma cell which produces the monoclonal
2   antibody of claim 35.

1   38.   The hybridoma cell line deposited as ATTC
2   #_____ .

1   39.   A method of identifying cells expressing LAM-
2   1, said method comprising
3   providing a sample of cells, at least some of which
4   are suspected of expressing LAM-1;
5   contacting said cells with a reagent comprising the
6   monoclonal antibody of claim 35; and
7   determining which cells form an immune complex with
8   said reagent.

1   40.   A method of isolating cells expressing LAM-1,
2   said method comprising
3   providing a sample of cells, at least some of which
4   are suspected of expressing LAM-1;
5   contacting said cells with the monoclonal antibody
6   of claim 35; and
7   separating those cells which have formed an immune
8   complex with said monoclonal antibody, from those cells
9   which have not.

1   41.   The method of claim 39, wherein said monoclonal
2   antibody is anti-LAM1-6 monoclonal antibody.

1   50.   The method of claim 46, wherein said monoclonal
2 antibody is a chimerized antibody having a variable region
3 derived from anti-LAM1-6 monoclonal antibody and constant
4 regions derived from a human antibody.

1   51.   A method of determining the degree of
2 expression of LAM-1 in a sample of leukocytes, said method
3 comprising
4   contacting a sample of leukocytes with the antibody
5 of claim 35, and
6   determining the level of immune complex formation in
7 said sample, said level being indicative of the degree of
8 expression of LAM-1 on said leukocytes.

1   52.   A monoclonal antibody which recognizes the LAM-
2 1 epitope recognized by anti-LAM1-7 monoclonal antibody.

1   53.   The monoclonal antibody of claim 52, wherein
2 said monoclonal antibody is anti-LAM1-7 monoclonal antibody.

1   54.   A hybridoma cell which produces the monoclonal
2 antibody of claim 52.

1   55.   The hybridoma cell line deposited as ATTC
2 #_____.

1   56.   A method of identifying cells expressing LAM-
2 1, said method comprising
3   providing a sample of cells, at least some of which
4 are suspected of expressing LAM-1;
5   contacting said cells with a reagent comprising the
6 monoclonal antibody of claim 52; and

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1   62.   The method of claim 61, wherein said monoclonal
2   antibody is anti-LAM1-7 monoclonal antibody.

1   63.   The method of claim 61, wherein said animal is
2   a human.

1   64.   The method of claim 61, wherein said animal is
2   suffering from inflammation.

1   65.   The method of claim 61, wherein said animal is
2   suffering from an autoimmune response.

1   66.   The method of claim 61, wherein said animal is
2   suffering from rejection of an organ or tissue transplant.

1   67.   The method of claim 63, wherein said monoclonal
2   antibody is a chimerized antibody having a variable region
3   derived from anti-LAM1-7 monoclonal antibody and constant
4   regions derived from a human antibody.

1   68.   A method of determining the degree of
2   expression of LAM-1 in a sample of leukocytes, said method
3   comprising
4   contacting a sample of leukocytes with the antibody
5   of claim 52, and
6   determining the level of immune complex formation in
7   said sample, said level being indicative of the degree of
8   expression of LAM-1 on said leukocytes.

1   69.   A monoclonal antibody which recognizes the LAM-
2   1 epitope recognized by anti-LAM1-8 monoclonal antibody.

3   obtaining a fluid sample from said animal;
4   contacting said sample with the monoclonal antibody
5 of claim 69; and
6   detecting formation of immune complexes formed by
7 said monoclonal antibody, said immune complexes indicating
8 the presence of LAM-1 shed from activated leukocytes.

1   77.   The method of claim 76, wherein said monoclonal
2 antibody is anti-LAM1-8 monoclonal antibody.

1   78.   A method of blocking leukocyte interactions
2 with endothelium in an animal, said method comprising
3 administering an effective amount of the monoclonal antibody
4 or fragment thereof of claim 69 to said animal.

1   79.   The method of claim 78, wherein said monoclonal
2 antibody is anti-LAM1-8 monoclonal antibody.

1   80.   The method of claim 78, wherein said animal is
2 a human.

1   81.   The method of claim 78, wherein said animal is
2 suffering from inflammation.

1   82.   The method of claim 78, wherein said animal is
2 suffering from an autoimmune response.

1   83.   The method of claim 78, wherein said animal is
2 suffering from rejection of an organ or tissue transplant.

1   84.   The method of claim 80, wherein said monoclonal
2 antibody is a chimerized antibody having a variable region

1   91.   A method of isolating cells expressing LAM-1,
2   said method comprising
3   providing a sample of cells, at least some of which
4   are suspected of expressing LAM-1;
5   contacting said cells with the monoclonal antibody
6   of claim 86; and
7   separating those cells which have formed an immune
8   complex with said monoclonal antibody, from those cells
9   which have not.

1   92.   The method of claim 90, wherein said monoclonal
2   antibody is anti-LAM1-9 monoclonal antibody.

1   93.   A method of detecting leukocyte activation in
2   an animal, said method comprising
3   obtaining a fluid sample from said animal;
4   contacting said sample with the monoclonal antibody
5   of claim 86; and
6   detecting formation of immune complexes formed by
7   said monoclonal antibody, said immune complexes indicating
8   the presence of LAM-1 shed from activated leukocytes.

1   94.   The method of claim 93, wherein said monoclonal
2   antibody is anti-LAM1-9 monoclonal antibody.

1   95.   A method of blocking leukocyte interactions
2   with endothelium in an animal, said method comprising
3   administering an effective amount of the monoclonal antibody
4   or fragment thereof of claim 86 to said animal.

1   96.   The method of claim 95, wherein said monoclonal
2   antibody is anti-LAM1-9 monoclonal antibody.

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1   105. A hybridoma cell which produces the monoclonal
2 antibody of claim 103.

1   106. The hybridoma cell line deposited as ATTC
2 #_____.

1   107. A method of identifying cells expressing LAM-
2 1, said method comprising
3   providing a sample of cells, at least some of which
4 are suspected of expressing LAM-1;
5   contacting said cells with a reagent comprising the
6 monoclonal antibody of claim 103; and
7   determining which cells form an immune complex with
8 said reagent.

1   108. A method of isolating cells expressing LAM-1,
2 said method comprising
3   providing a sample of cells, at least some of which
4 are suspected of expressing LAM-1;
5   contacting said cells with the monoclonal antibody
6 of claim 103; and
7   separating those cells which have formed an immune
8 complex with said monoclonal antibody, from those cells
9 which have not.

1   109. The method of claim 107, wherein said
2 monoclonal antibody is anti-LAM1-10 monoclonal antibody.

1   110. A method of detecting leukocyte activation in
2 an animal, said method comprising
3   obtaining a fluid sample from said animal;
4   contacting said sample with the monoclonal antibody
5 of claim 103; and

1   119. A method of determining the degree of
2   expression of LAM-1 in a sample of leukocytes, said method
3   comprising
4   contacting a sample of leukocytes with the antibody
5   of claim 103, and
6   determining the level of immune complex formation in
7   said sample, said level being indicative of the degree of
8   expression of LAM-1 on said leukocytes.

1   120. A monoclonal antibody which recognizes the LAM-
2   1 epitope recognized by anti-LAM1-11 monoclonal antibody.

1   121. The monoclonal antibody of claim 120, wherein
2   said monoclonal antibody is anti-LAM1-11 monoclonal
3   antibody.

1   122. A hybridoma cell which produces the monoclonal
2   antibody of claim 120.

1   123. The hybridoma cell line deposited as ATTC
2   #_____.

1   124. A method of identifying cells expressing LAM-
2   1, said method comprising
3   providing a sample of cells, at least some of which
4   are suspected of expressing LAM-1;
5   contacting said cells with a reagent comprising the
6   monoclonal antibody of claim 120; and
7   determining which cells form an immune complex with
8   said reagent.

1   125. A method of isolating cells expressing LAM-1,
2   said method comprising

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1   132. The method of claim 129, wherein said animal is
2 suffering from inflammation.

1   133. The method of claim 129, wherein said animal is
2 suffering from an autoimmune response.

1   134. The method of claim 129, wherein said animal is
2 suffering from rejection of an organ or tissue transplant.

1   135. The method of claim 131, wherein said
2 monoclonal antibody is a chimerized antibody having a
3 variable region derived from anti-LAM1-11 monoclonal
4 antibody and constant regions derived from a human antibody.

1   136. A method of determining the degree of
2 expression of LAM-1 in a sample of leukocytes, said method
3 comprising
4   contacting a sample of leukocytes with the antibody
5 of claim 120, and
6   determining the level of immune complex formation in
7 said sample, said level being indicative of the degree of
8 expression of LAM-1 on said leukocytes.

1   137. A monoclonal antibody which recognizes the LAM-
2 1 epitope recognized by anti-LAM1-14 monoclonal antibody.

1   138. The monoclonal antibody of claim 137, wherein
2 said monoclonal antibody is anti-LAM1-14 monoclonal
3 antibody.

1   139. A hybridoma cell which produces the monoclonal
2 antibody of claim 137.

6   detecting formation of immune complexes formed by
7   said monoclonal antibody, said immune complexes indicating
8   the presence of LAM-1 shed from activated leukocytes.

1   145. The method of claim 144, wherein said
2   monoclonal antibody is anti-LAM1-14 monoclonal antibody.

1   146. A method of blocking leukocyte interactions
2   with endothelium in an animal, said method comprising
3   administering an effective amount of the monoclonal antibody
4   or fragment thereof of claim 137 to said animal.

1   147. The method of claim 146, wherein said
2   monoclonal antibody is anti-LAM1-14 monoclonal antibody.

1   148. The method of claim 146, wherein said animal is
2   a human.

1   149. The method of claim 146, wherein said animal is
2   suffering from inflammation.

1   150. The method of claim 146, wherein said animal is
2   suffering from an autoimmune response.

1   151. The method of claim 146, wherein said animal is
2   suffering from rejection of an organ or tissue transplant.

1   152. The method of claim 148, wherein said
2   monoclonal antibody is a chimerized antibody having a
3   variable region derived from anti-LAM1-14 monoclonal
4   antibody and constant regions derived from a human antibody.

FIG. IA

FIG. IB

FIG. 2A

```
                                                              1
                                                              M   G
gaattcCCCTTT GGGCAAGGAC CTGAGACCCT TGTGCTAAGTCAAGAGGCTCA ATG GGC

 C   R   T   R   E   G   P   S   K   A   M
                         10
TGC AGA ACT AGA GAA GGA CCA AGC AAA GCC ATG   94

 I   F   P   W   K   C   Q   S   T   Q   R   D   L   W   N   I
                         20                                    30
ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC

 F   K   L   W   G   W   T   M   L   C   C   D
                             40
TTC AAG TTG TGG GGG TGG ACA ATG CTC TGT TGT GAT   168

 F   L   A   H   G   T   D   C   W   T   Y   H   S   E
                     50
TTC CTG GCA CAT GGA ACC GAC TGC TGG ACT TAC CAT TAT TCT GAA

 K   P   M   N   W   Q   R   A   R   F   C
             60                          80
AAA CCC ATG AAC TGG CAA AGG GCT AGA AGA TTC TGC   262

 R   D   N   Y   T   D   L   V   A   I   Q   N   K   A   E   I
 CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA CAA AAC AAG GCG GAA ATT
                 90

 E   Y   L   E   K   T   L   P   F   S   R   S
GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT TCT   346
```

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           100                                      110
  Y   Y   W   I   G   I   R   K   I   G   G   I   W   T   W
 TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG
                                    120
  V   G   T   N   K   S   L   T   E   E   A   E   N
 GTG GGA ACC AAC AAA TCT CTC ACT GAA GAA GCA GAG AAC  430
                 130                                   140
  W   G   D   G   E   P   N   N   K   K   N   K   E   D   C
 TGG GGA GAT GGT GAG CCC AAC AAC AAG AAG AAC AAG GAG GAC TGC
                                150
  V   E   I   Y   I   K   R   N   K   D   A   G   K
 GTG GAG ATC TAT ATC AAG AGA AAC AAA GAT GCA GGC AAA  514
                         160
  W   N   D   D   A   C   H   K   L   K   A   A   L   C   Y
 TGG AAC GAT GAC GCC TGC CAC AAA CTA AAG GCA GCC CTC TGT TAC
170                                          180
  T   A   S   C   Q   P   W   S   C   S   G   H   G
 ACA GCT TCT TGC CAG CCC TGG TCA TGC AGT GGC CAT GGA  598
                                 190
  E   C   V   E   I   I   N   N   Y   T   C   N   C   D   V
 GAA TGT GTA GAA ATC ATC AAT AAT TAC ACC TGC AAC TGT GAT GTG
         200                                      210
  G   Y   Y   G   P   Q   C   Q   F   V   I   Q   C
 GGG TAC TAT GGG CCC CAG TGT CAG TTT GTG ATT CAG TGT  682
                                    220
  E   P   L   E   A   P   E   L   G   T   M   D   C   T   H
 GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC TGT ACT CAC
                     230
  P   L   G   N   F   N   F   N   S   Q   C   A   F
 CCT TTG GGA AAC TTC AAC TTC AAC TCA CAG TGT GCC TTC  766
     240                                      250
  S   C   S   E   G   T   N   L   T   G   I   E   E   T   T
 AGC TGC TCT GAA GGA ACA AAC TTA ACT GGG ATT GAA GAA ACC ACC
                         260
  C   E   P   F   G   N   W   S   S   P   E   P   T
 TGT GAA CCA TTT GGA AAC TGG TCA TCT CCA GAA CCA ACC  850
         270                                      280
  C   Q   V   I   Q   C   E   P   L   S   A   P   D   L   G
 TGT CAA GTG ATT CAG TGT GAG CCT CTA TCA GCA CCA GAT TTG GGG
                             290
  I   M   N   C   S   H   P   L   A   S   F   S   F
 ATC ATG AAC TGT AGC CAT CCC CTG GCC AGC TTC AGC TTT  934
                 300
  T   S   A   C   T   F   I   C   S   E   G   T   E   L   I
 ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA ACT GAG TTA ATT
310                                          320
  G   K   K   K   T   I   C   E   S   S   G   I   W
 GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA ATC TGG 1018
                             330
  S   N   P   S   P   I   C   Q   K   L   .D   K   S   F   S
 TCA AAT CCT AGT CCA ATA TGT CAA AAA TTG GAC AAA AGT TTC TCA
         340                                      350
  M   I   K   E   G   D   Y   N   P   L   F   I   P
 ATG ATT AAG GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA 1102
```

FIG. 2B

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```
                                                   360
      V    A    V    M    V    T    A    F    S    G    L    A    F    I    I
     GTG  GCA  GTC  ATG  GTT  ACT  GCA  TTC  TCT  GGG  TTG  GCA  TTT  ATC  ATT
                         370
      W    L    A    R    R    L    K    K    G    K    K    S    K
     TGG  CTG  GCA  AGG  AGA  TTA  AAA  AAA  GGC  AAG  AAA  TCC  AAG  1186
          380
      R    S    M    N    D    P    Y    *
     AGA  AGT  ATG  AAT  GAC  CCA  TAT  TAA  ATCGCCCTTG  GTGAAAGAAA
```

ATTCTTGGAA TACTAAAAAT CATGAGATCC TTTAAATCCT TCCATGAAAC 1280

GTTTTGTGTG GTGGCACCTC CTACGTCAAA .CATGAAGTGT GTTTCCTTCA

GTGCATCTGG GAAGATTTCT ACCTGACCAA GAGTTCCTTC AGCTTCCATT 1380

TCACCCCTCA TTTATCCCTC AACCCCCAGC CCACAGGTCT TTATACAGCT

CAGCTTTTTC TCTTTTCTGA GGAGAAACAA ATAACACCAT AAAGGGAAAG 1480

GATTCATGTG GAATATAAAG ATGGCTGACT TTGCTCTTTC TTGACTCTTG

TTTTCAGTTT CAATTCAGTG CTGTACTTGA TGACAGACAC TTCTAAATGA 1580

AGTGCAAATT TGATACATAT GTGAATATGG ACTCAGTTTT CTTGCAGATC

AAATTTCGCG TCGTCTTCTG TATACGTCCA GGTACACTCT ATGAAGTCAA 1680

AAGTCTACGC TCTCCTTTCT TTCTAACTCC AGTGAAGTAA TGGGGTCCTG

CTCAAGTTGA AAGAGTCCTA TTTGCACTGT AGCCTCGCCG TCTGTGAATT 1780

GGACCATCCT ATTTAACTGG CTTCAGCCTC CCCACCTTCT TCAGCCACCT

CTCTTTTTCA GTTGGCTGAC TTCCACACCT AGCATCTCAT GAGTGCCAAG 1880

CAAAAGGAGA GAAGAGAGAA ATAGCCTCCG CTGTTTTTTA GTTTGGGGGT

TTTGCTGTTT CCTTTTATGA GACCCATTCC TATTTCTTAT AGTCAATGTT 1980

TCTTTTATCA CGATATTATT AGTAAGAAAA CATCACTGAA ATGCTAGCTG

CAACTGACAT CTCTTTGATG TCATATGGAA GAGTTAAAAC AGGTGGAGAA 2080

ATTCCTTGAT .TCACAATGAA ATGCTCTCCT TTCCCCTGCC CCCAGACCTT

TTATCCACTT ACCTAGATTC TACATATTCT TTAAATTTCA TCTCAGGCCT 2180

CCCTCAACCC CACCACTTCT TTTATAACTA GTCCTTTACT AATCCAACCC

ATGATGAGCT CCTCTTCCTG GCTTCTTACT GAAAGGTTAC CCTGTAACAT 2280

GCAATTTTGC ATTTGAATAA AGCCTGCTTT TTAAGTGTTA AAAAgaattc 2330

FIG. 2C

FIG. 3A

```
LAM-1    35  GMWMLCC-DFLAHHGTDCWTYHYSEKPMNWQRARRFCRNDYTDLVAIQN-
FcE-R   175  GFVCNTCPEKWINFQRKC--MYPGLGTKQWVHARYACDDMEGQLVSIHS-
C-HL     75  LFPCGAQSRQWEYFEGRC--MYFSLSRMSWHKAKEECEEMHSHLIILDS-
H-MBP   118  NGTYQKCTSLGKQVNLFFLTNGE-IMTFELVLALC-VKFQPLWPPPG-
F-PGC   452  GQDTETCDYGWHKFQGQC--MKYFAHRRTWDAAERECRLQGAHLTSILS-
HHL-1   148  GSERTCCPVNWVEHERSC--YMPSRSGKAWADADNYCRLENAHLVAVTS-
ISL      17  IFISTAAVPQLQKALDGREYLIETELKYTNWHQAWHECARHDQLVTIESA

LAM-1        --KAEIEYLEKTLPFSRSYYWIGIRKIG---GIWMIWVGTNKSLTEEAAENW
FcE-R        --PEE-QDFLTLHASHTGSWIGLRNLDLK---GEFIWVDGSHVD---YSNW
C-HL         --YAK-QNFVMFRTRNERFWIGLTDENQE---GEWQWVDGTDTRSS--FTFW
H-MBP        --MAA-EKGAIQNLILEEAFLGMPDELTE---GQF-VDLTGNRLT--YTNW
F-PGC        --HEE-QMFVNRVGHDYQ-WIGLNDKMFE---HDFRWTDGS-----TLQYENW
HHL-1        --WEE-QKPVQHHIGPVNTMGLHDQN-----GPWKWVDGTDYE-TG-PKNW
ISL          DKNNAIIDLMKRVVGKSHNLWLGGNDEYSRDYGPFFWS-PLGQAFS--FAYW

LAM-1   160  GDGEPNNKKNK----------EDCVEIYIKRNKDAGKWNDDACH-KLKAAICYT 160
FcE-R   284  APGEPTSRSQ---G--EDCVMM----R----GSCRWNDAFCDRKLGAWVCDR 284
C-HL    203  LEGEPNNR-----GFNEDCAH-------VWTSGQWNDVYCTYECYY-VCEL 203
H-MBP   245  NEGEPNNA-----GSDEHCVL--------LIKNGQWNDSPCF-HTPSAVCEF 245
F-PGC   580  RPNQPDSFFSA--G----EDCVVII----WHENGQWNDVPCNYHLITY-ICKK 580
HHL-1   279  RPEQPDDWYGHGLGGGEDCAH-------PTDDGRWNDDVCQ--RPYRWVCETI 279
ISL     159  SENNPNNYLHQ----EFCVIWNTLPLY--QWNDDDCN-VLMGYICEP 159
```

FIG. 3B

```
                                                                      *
LAM-1  173  CQP---WSCSGHGECVEIINN--YTCNCDVGYYGPQCQ  205
EGF      6  CPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQ   43
F-IX    51  CES--NPCLNGGSCKDDINS-YECWCPFGFEGKNCE     83
F-PGCP 382  CKM---NPCKNGGTCYPTETS-YVCTCVPGYSGDQCE   414
```

```
            *                                              +         +
LAM-1  207  VIQCEPLEAPELGTMDCTHPLGNFNFN-SQCAF---
LAM-1  269  VIQCEPLSAPDLGIMNCSHPLASFSFT-SACTF---
Ba     137  AGYCSNPGIP-IGTRKVGSQYRLED--SV-TY---
CR1    694  V--CQPPEILH-GEHTPSHQD-NFS-PGQEVFY---
IL-2R  101  PGHCREPP-PWENEATERIYHFVVG-QMVYYQ----
F-XIII   1  EKPCGFPHVENGRIAQYYYTFKSFYEPMSIDKKLSF
```

FIG. 3C

```
            *          *          *
LAM-1  268  SCSEGTN-L-TGIEE-TTC----EPFGNWSSPEPTICQ  268
LAM-1  330  ICSEGTE-L-IGKKK-TIC----ESSGIWSNPSPICQ   330
Ba     194  HCSRGLT-L-RGSQR-RTC----QEGGSWSGTEPSCQ   194
CR1    751  SCEPGYD-L-RGAAS-LHC----TPQGDWSDEAPRCA   751
IL-2R  164  -CVQGYRALHRGPAE-SVCKMTHGKTRWTQPQLICT    164
F-XIII  68  FCLAGYTTESSRQEEQTTCT-TE--WS-PEPRCF       68
```
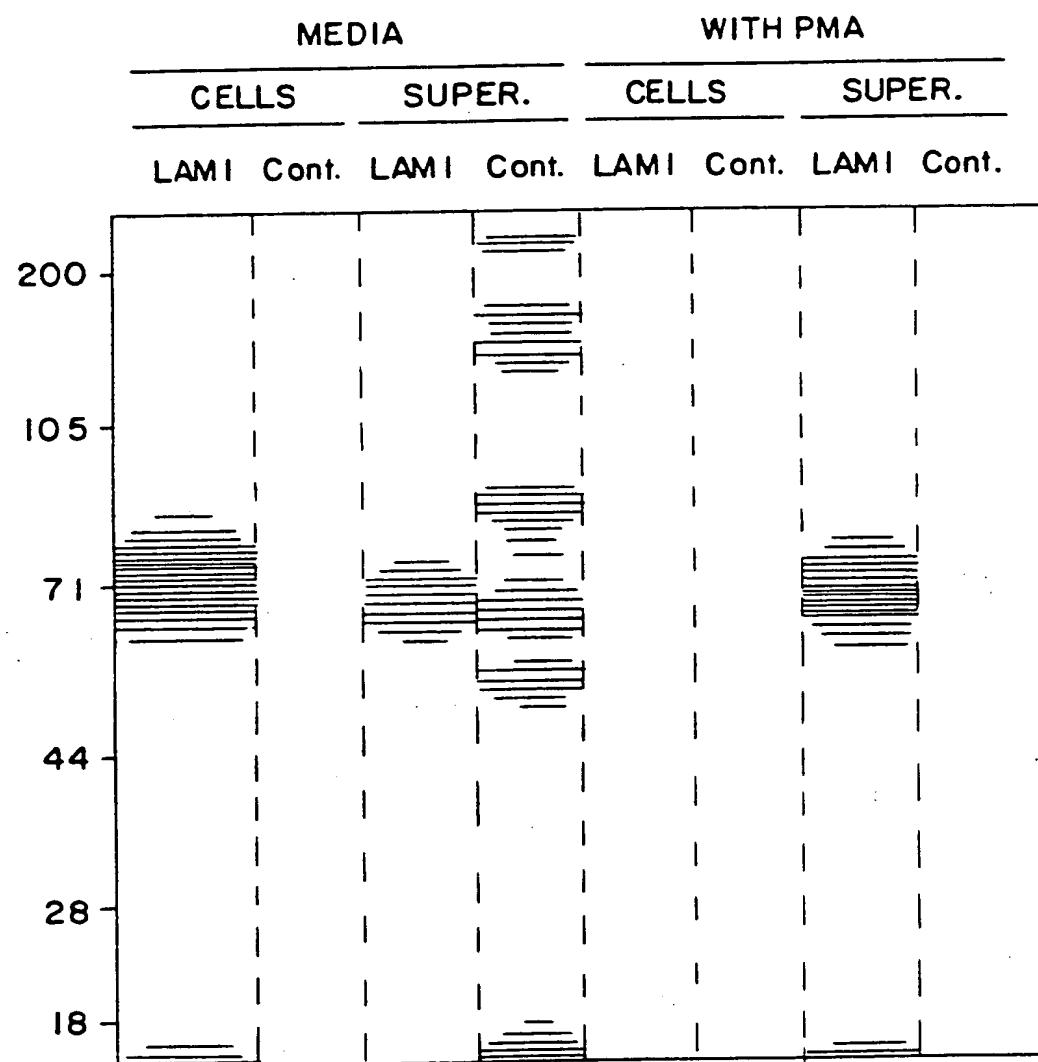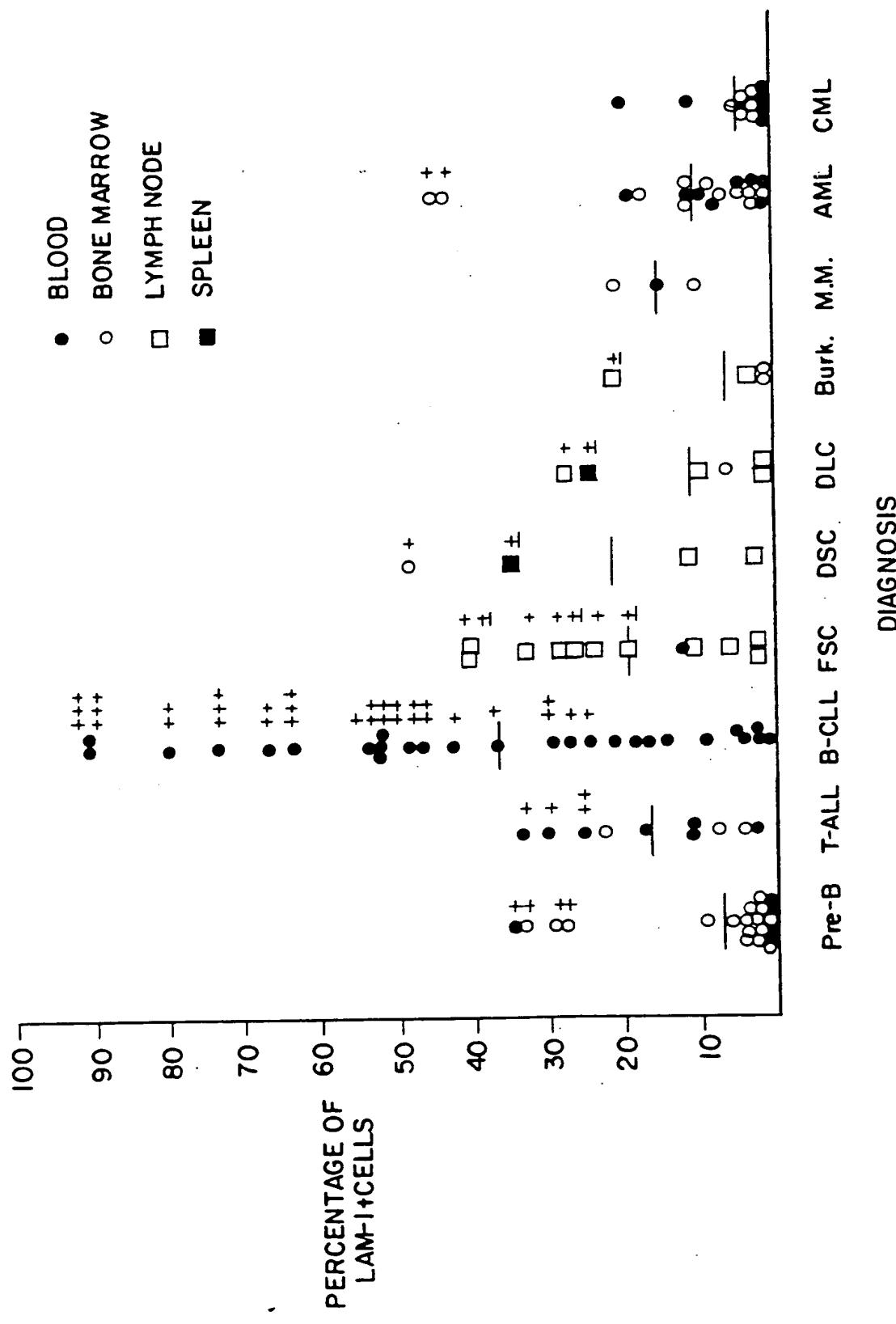
FIG. 4

FIG. 5

FIG. 6

ANTI-LAM 1.3 ANTIBODY   PPME-FITC BINDING

FIG. 7

FLUORESCENCE INTENSITY (LOG SCALE)

FIG.8

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FIG. 9

FIG. 10

FIG. II
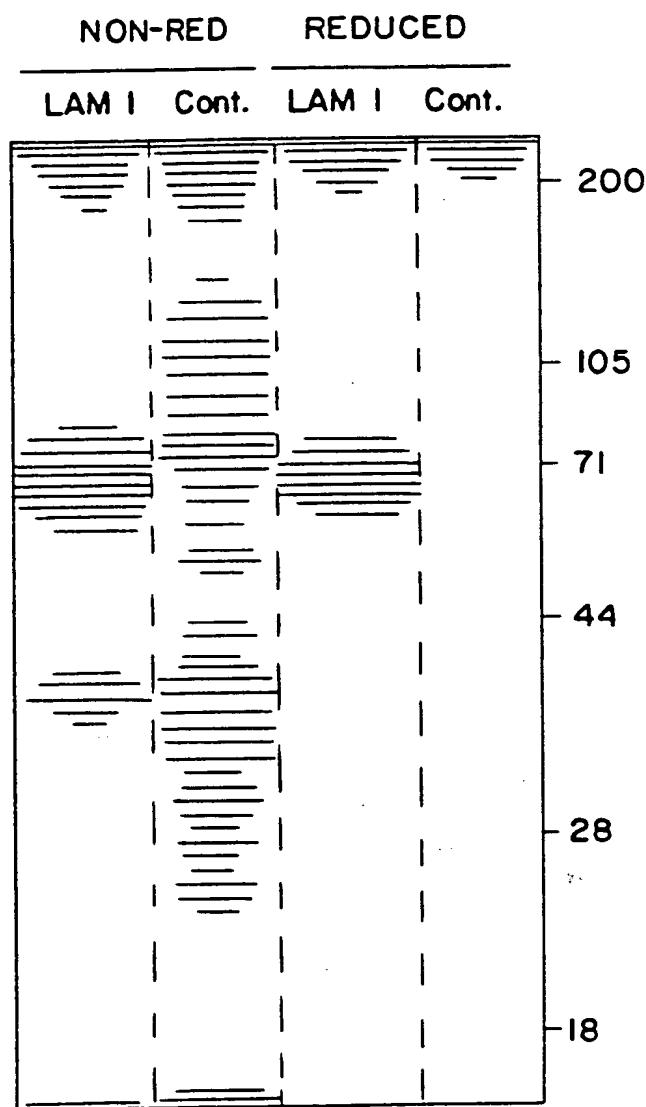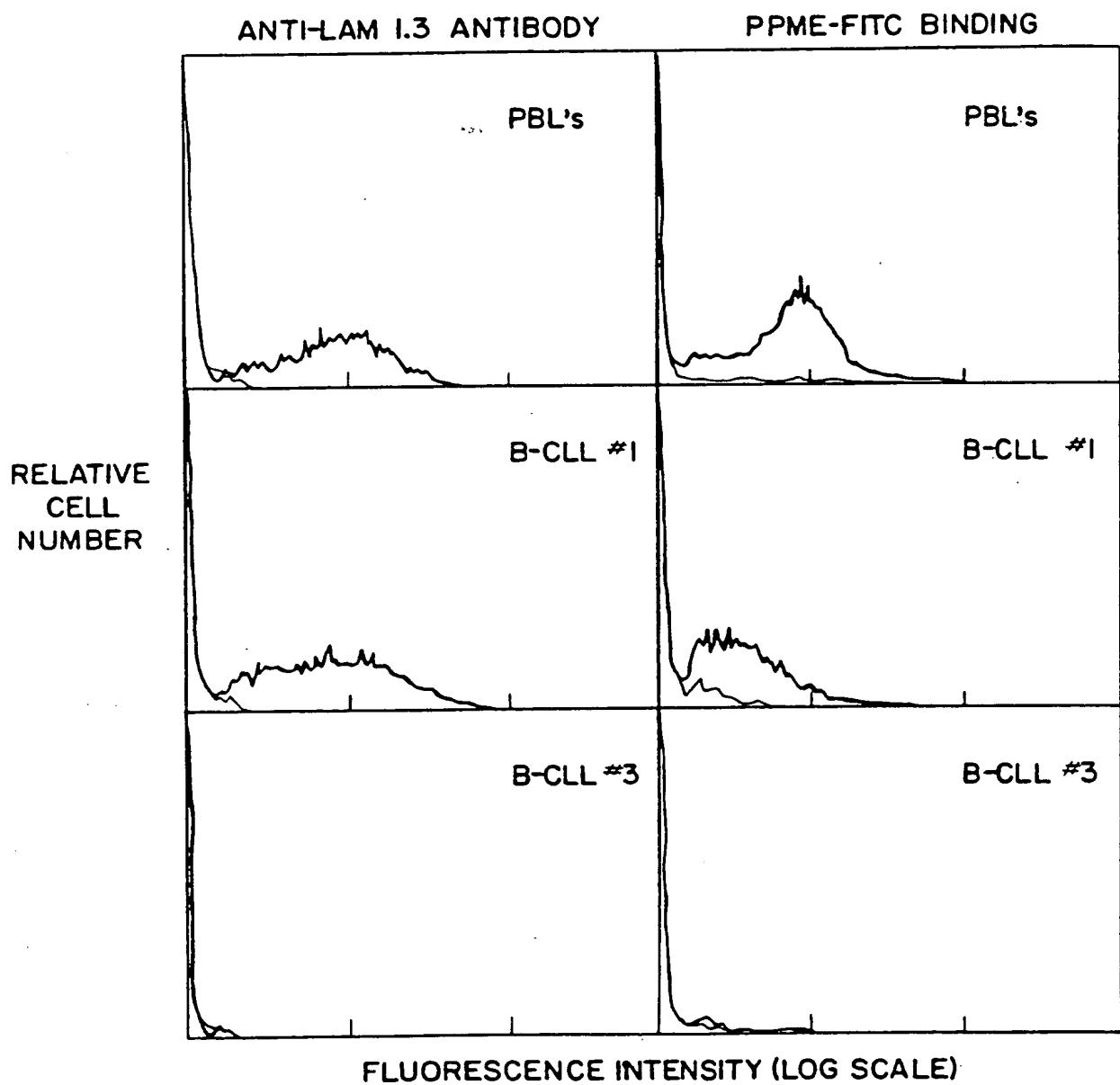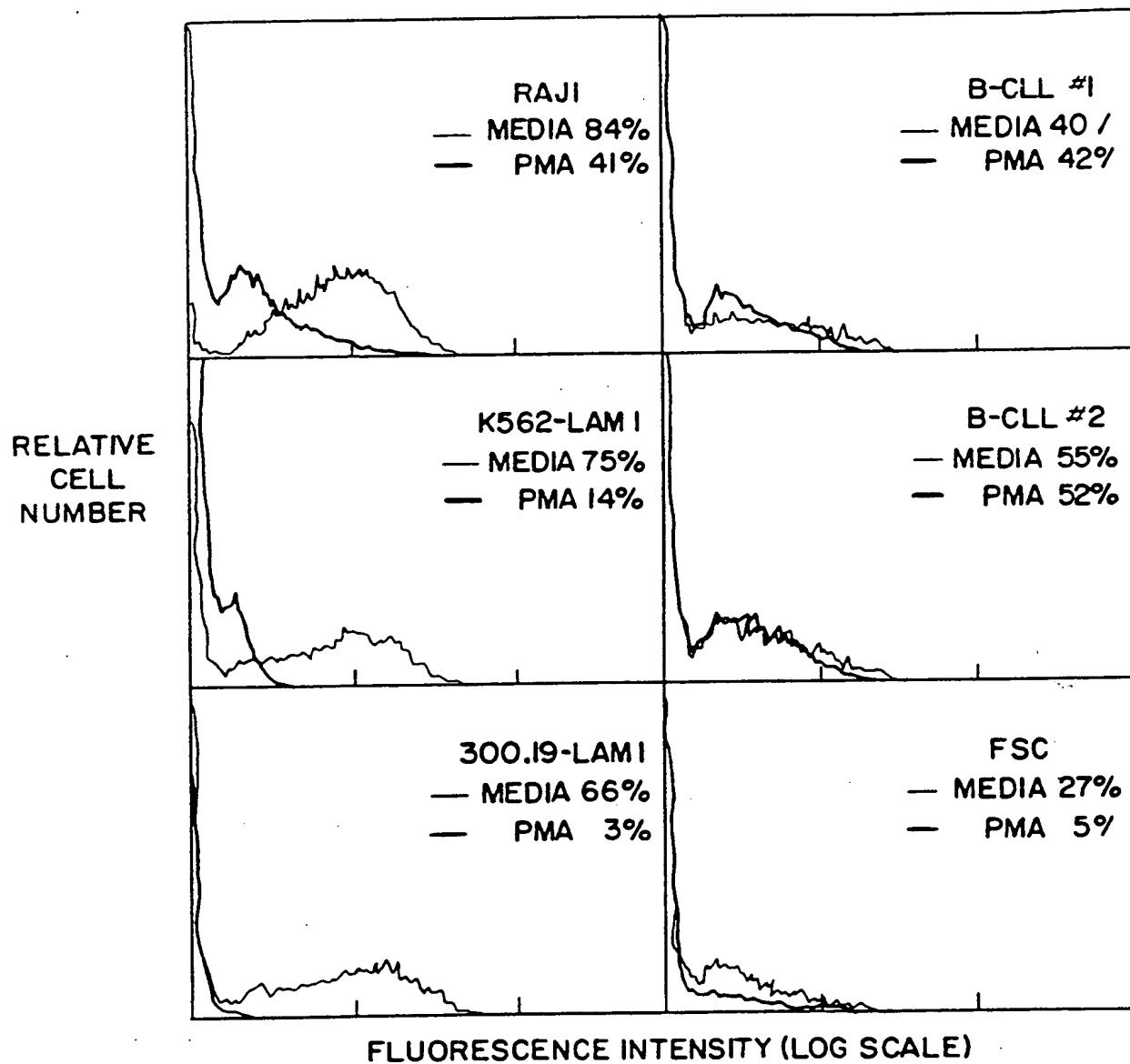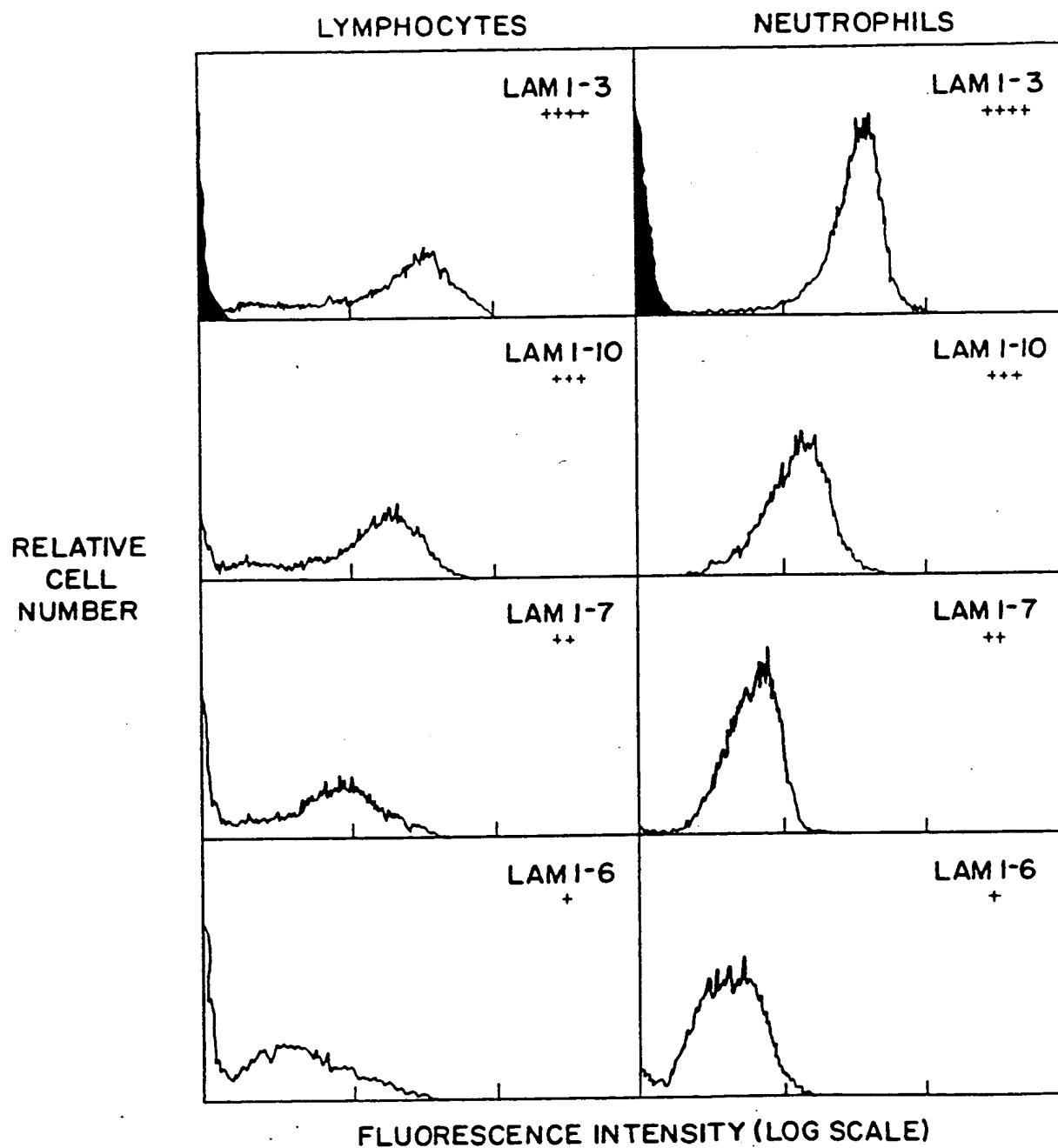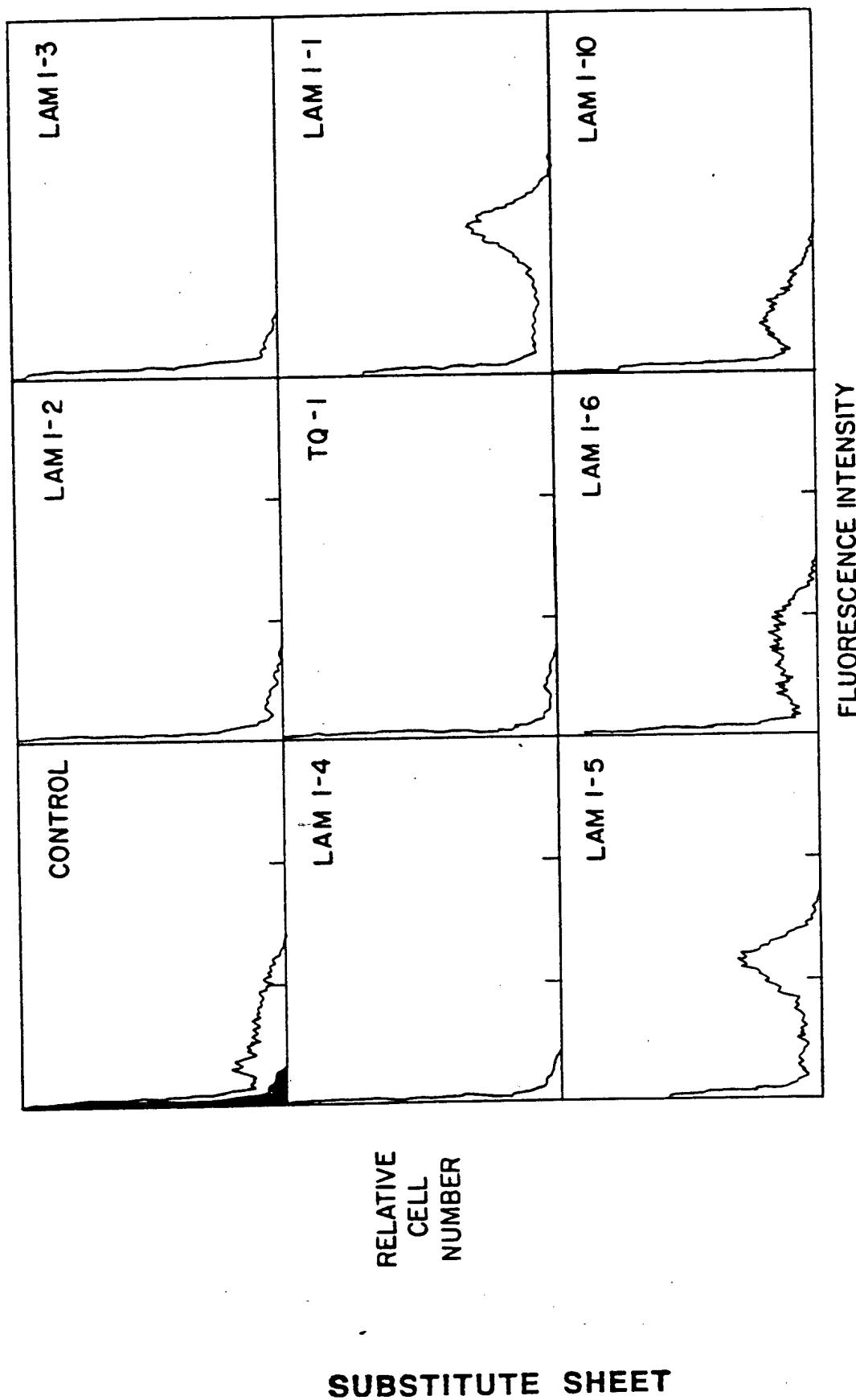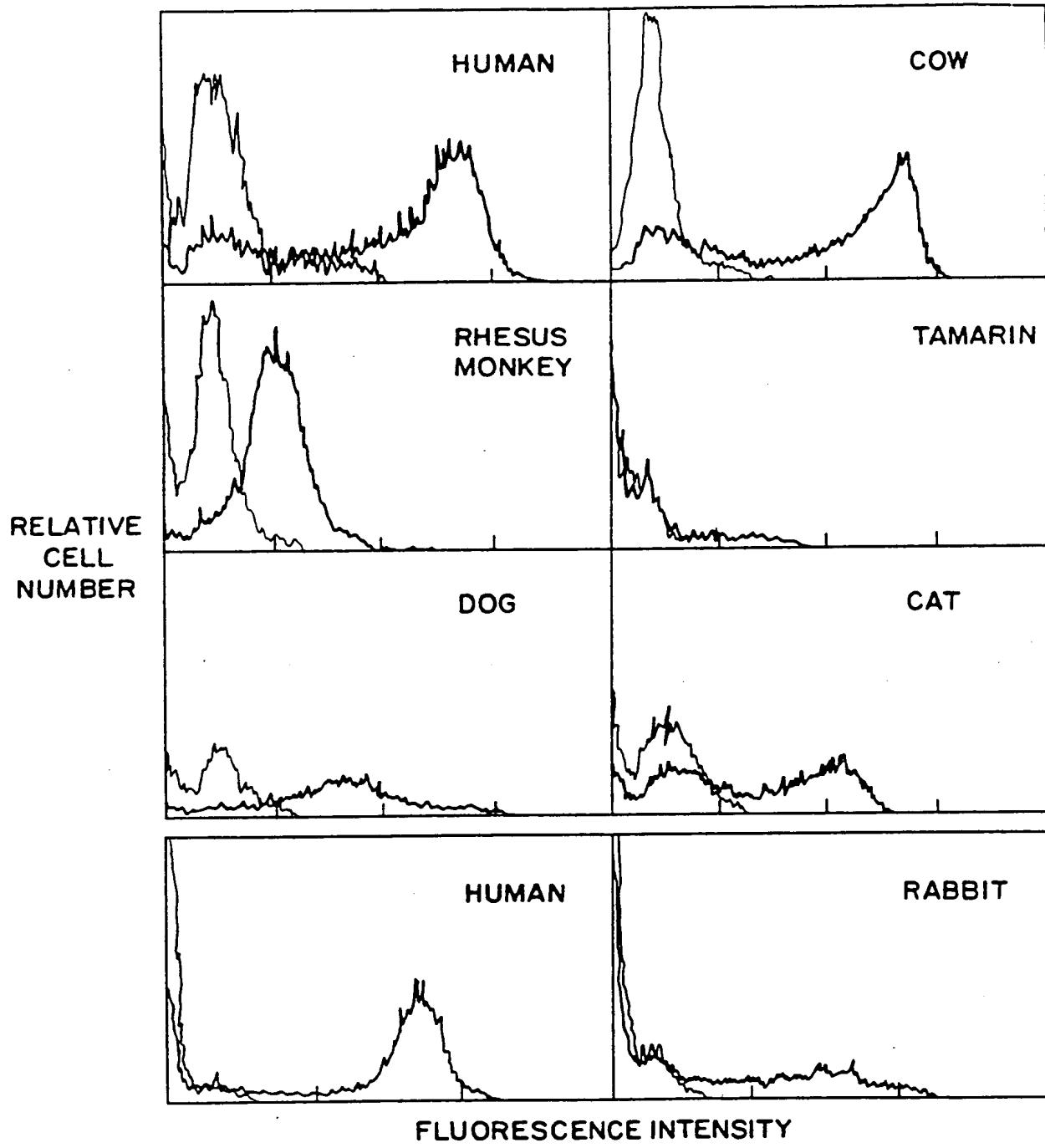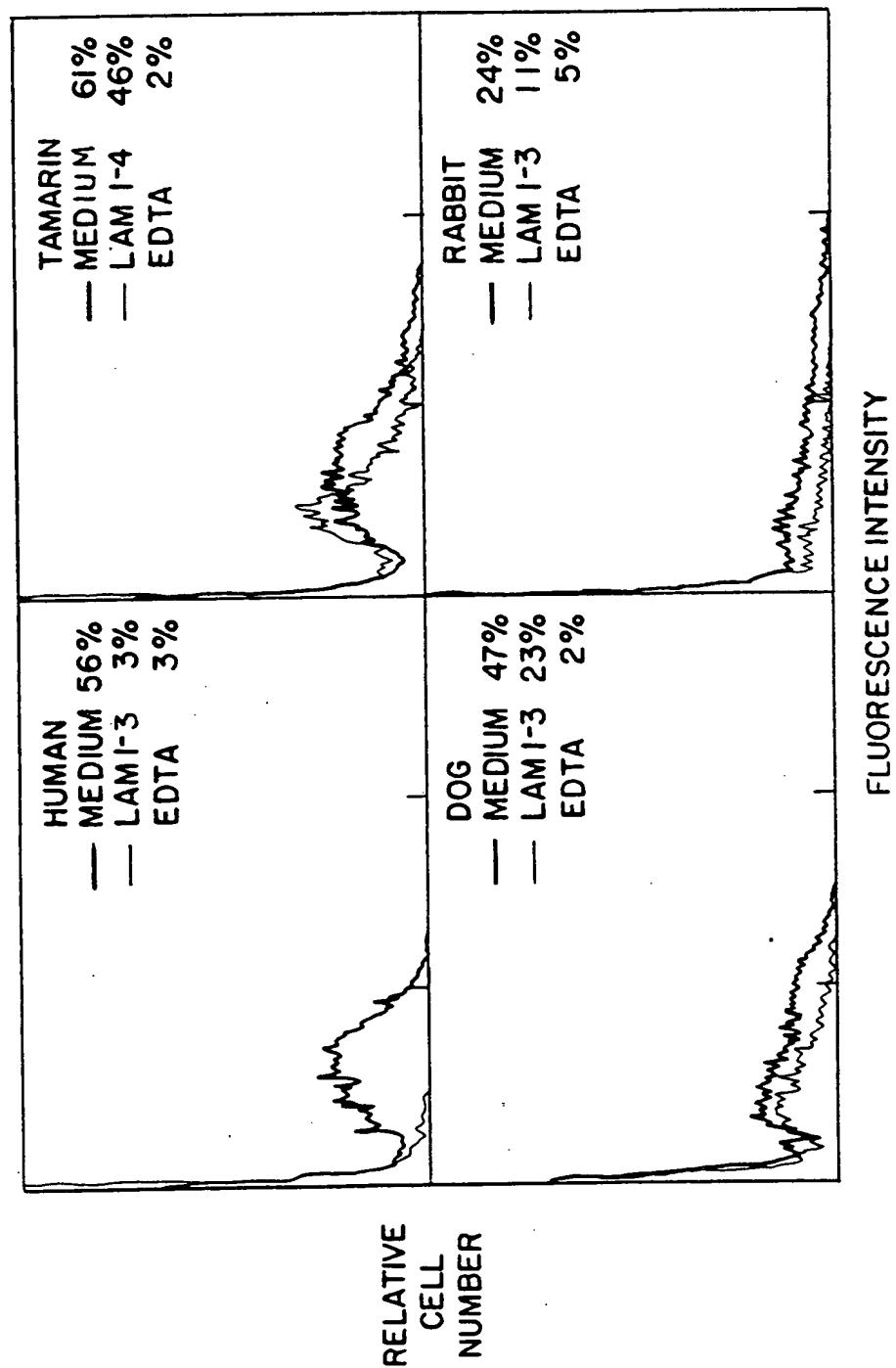
FIG. 12

FUNCTIONAL AND STRUCTURAL REGIONS OF LAM-1
IDENTIFIED BY MONOCLONAL ANTIBODIES
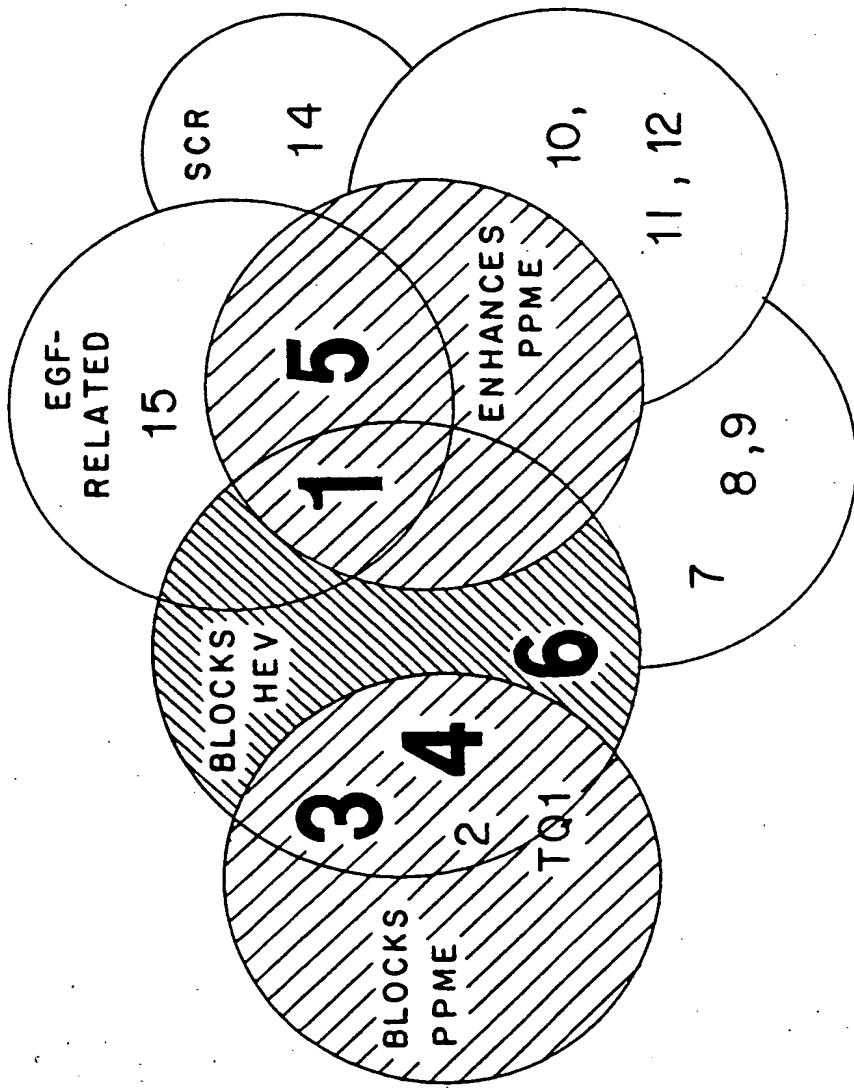
FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/06127

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/04, 39/00; C07K 13/00, 15/28; C12N 15/00, C12P 21/08

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 435/70.21, 172.2; 530/387.1, 388.1, 388.22, 388.24, 388.85, 866, 867

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, SCISEARCH, WPI, APS

SEARCH TERMS: LAM 1, ANTIBODY, TEDDER

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF IMMUNOLOGY, Volume 128, No. 1, issued 01 January 1982, E.L. Reinherz et al., "Heterogeneity of Human T4+ Inducer T cells Defined by a Monoclonal Antibody that Delineates Two Functional Subpopulations", pages 463-468, see entire document.	1-153
Y	PROC. NATL. ACAD. SCI. USA, Volume 87, issued March 1990, Kishimoto et al. "Identification of a Human Peripheral Lymph Node Homing Receptor: A Rapidly Down-Regulated Adhesion Molecule", pages 2244-2248, see entire document.	1-153
Y	JOURNAL OF CELL BIOLOGY, Volume 107, issued November 1988, N.W. Wu et al., "Evolutionary Conservation of Tissue-specific Lymphocyte-Endothelial Cell Recognition Mechanisms Involved in Lymphocyte Homing", pages 1845-1851, entire document.	1-153
Y	JOURNAL OF IMMUNOLOGY, Volume 144, issued 15 January 1990, T.F. Tedder, "Expression of the Human Leukocyte Adhesion Molecule, LAM1: Identity with the TQ1 and Leu-8 Differentiation Antigens", pages 532-540, see entire document.	1-153

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 SEPTEMBER 1992	1 6 SEP 1992

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF EXPERIMENTAL MEDICINE. Volume 170. issued 01 July 1989, T.F. Tedder et al., "Isolation and Chromosomal Localization of cDNAs Encoding a Novel Human Lymphocyte Cell Surface Molecule, LAM-1", pages 123-133, see entire document.	1-153
Y	JOURNAL OF CELL BIOLOGY, Volume 109. issued July 1989, B.R. Bowen et al., "Characterization of a Human Homologue of the Murine Peripheral Lymph Node Homing Receptor", pages 421-427, see entire document.	1-153
Y	USA 5,098,833 (Lasky et al.) 24 March 1992, see entire document.	1-153
Y	CELL, Volume 56, issued 24 March 1989, Stoolman, "Adhesion Molecules Controlling Lymphocyte Migration", pages 907-910, see entire document.	1-153
Y	CELL, Volume 62. issued 13 July 1990, Osborn, "Leukocyte Adhesion to Endothelium in Inflammation", pages 3-6, entire document.	1-153

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/85.8; 435/70.21, 172.2; 530/387.1, 388.1, 388.22, 388.24, 388.85, 866, 867

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